COMPARATIVE POPULATION STRUCTURE OF THE CARIBBEAN SEA FAN CORAL *GORGONIA VENTALINA*, AND ITS DINOFLAGELLATE ENDOSYMBIONT, *SYMBIODINIUM* SP.

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COMPARATIVE POPULATION STRUCTURE OF THE CARIBBEAN SEA FAN CORAL *GORGONIA VENTALINA*, AND ITS DINOFLAGELLATE ENDOSYMBIONT, *SYMBIODINIUM* SP.

Jason Phillip Andras, Ph.D.

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The high productivity of tropical reefs is largely due to the symbiosis between corals and photosynthetic dinoflagellates of the genus *Symbiodinium*. The resilience of the coral holobiont depends on the genetic diversity and connectivity of both symbiotic partners. However, little is known regarding the population structure of most corals or the symbionts they host. Here, I describe and compare the range-wide population genetic structure of an ecologically dominant Caribbean octocoral, *Gorgonia ventalina*, and its dinoflagellate symbiont, *Symbiodinium* B1/184. Twenty polymorphic microsatellite markers (10 for *G. ventalina*, 10 for *Symbiodinium*) were used to quantify genetic diversity and differentiation of both symbiotic partners at 35 localities, spanning 3124 km across the Caribbean and Western Atlantic. Both *G. ventalina* and its symbiont were significantly differentiated among most localities, across distances as short as one kilometer, and estimated migration rates indicate that both symbiotic partners are demographically independent at the regional scale. Patterns of population differentiation for *G. ventalina* and its *Symbiodinium* corroborated previously reported biogeographic breaks (e.g. an east/west divide near Puerto Rico) and identified several previously unreported junctures (e.g. the Yucatan Channel and the Florida Strait). Across the range, genetic differentiation was positively correlated with geographic distance for both partners, suggesting that

distance is an important isolating mechanism. Differentiation of *G. ventalina* was also negatively correlated with dispersal probability as estimated by an oceanographic model, indicating that ocean currents are an important means of connectivity. Although the population structures of *G. ventalina* and its *Sybiodinium* were similar, they were not completely congruent, indicating that dispersal of these partners is not coupled and that symbiont transmission occurs horizontally. The *Symbiodinium* haplotype hosted by some *G. ventalina* colonies changed in response to experimental heat or light stress or reciprocal transplant across depth, suggesting that fine-scale *Symbiodinium* diversity corresponds with functional diversity, and stress-related mortality was lower among *G. ventalina* colonies that changed symbionts than those that did not. Taken together, these results indicate that populations of *G. ventalina* and *Symbiodinium* are structured independently across relatively short distances, and that some *G. ventalina* colonies may change their *Symbiodinium* as a potentially "adaptive" response to stress.

BIOGRAPHICAL SKETCH

Jason Phillip Andras was born on May 7th, 1977 in Hazleton, Pennsylvania. His interest in the biological sciences grew from his love for the natural world, which was fostered by a childhood spent outdoors. Jason pursued Bachelor's of Science degrees with Honors in Biology and Chemistry at the Pennsylvania State University. His undergraduate Honors thesis, based on research conducted under the advisement of Dr. Chuck Fisher, investigated the mechanisms used by vestimentiferan tubeworms residing at hydrocarbon seeps to obtain sulfide for their chemoautotrophic bacterial symbionts. After completing his undergraduate degrees, Jason was awarded a Fulbright Fellowship to pursue research in the laboratory of Dr. Ove Hoegh-Guldberg at the University of Queensland in Brisbane, Australia. There, his research focused on the physiological and biochemical responses of corals and their algal symbionts to thermal stress. Intrigued by the biology of the coral/algal symbiosis and beguiled by the beauty of tropical reefs, Jason decided to continue studying coral symbioses in the laboratory of Dr. Drew Harvell at Cornell University. With the guidance of Dr. Harvell and the other members of his doctoral committee, Drs. Rick Harrison, Monica Geber, and Jim Morin, Jason developed an independent research program on the comparative population genetics of the Caribbean sea fan coral, *Gorgonia ventalina*, and its algal symbiont. For his postdoctoral research, Jason decided to shift his focus to the parasitic end of the symbiotic spectrum. He is currently working as a postdoctoral fellow in the laboratory of Dr. Dieter Ebert at the University of Basel, Switzerland, where his research will focus on the evolution and ecology of the water flea, *Daphnia magna*, and its bacterial parasite, *Pasteuria ramosa*.

To my wife and best friend, Kate.

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CHAPTER 1:

Isolation and characterization of microsatellite loci in the Caribbean sea fan coral, *Gorgonia ventalina*

ABSTRACT

Here we report primers for ten microsatellite loci from the Caribbean sea fan coral, *Gorgonia ventalina*. Primers were tested on 237 genomic DNA extracts taken directly from tissue samples of *G. ventalina*. All loci were polymorphic with allelic richness ranging from 4 to 52. Expected heterozygosity ranged from 0.14 to 0.96. Preliminary data suggest that these microsatellites will be useful tools for studies of the population genetics of this important Caribbean coral species.

INTRODUCTION

Gorgonian corals are conspicuous benthic inhabitants of most healthy Caribbean reefs (Opresko 1973, Yoshioka and Yoshioka 1989, Sanchez et al. 1997). Their high abundance and complex three-dimensional structure provide essential habitat for a diversity of reef fauna, making them important ecosystem structuring organisms. One of the most abundant Caribbean gorgonian species is the sea fan coral, *Gorgonia ventalina* (Ward et al. 2006). Over the past two decades, populations of *G. ventalina* have been heavily impacted by the fungal disease aspergillosis (Nagelkerken et al. 1997, Kim and Harvell 2004, Kim et al. 2006), and numerous studies have investigated the ecology, epidemiology, and immunology of this coral disease system. Yet most basic information regarding the life history and population biology of *G. ventalina* remains unknown. An understanding of the distribution and dynamics of population genetic diversity is essential for effective management and conservation, particularly for species such as *G. ventalina* that have been heavily

impacted by anthropogenic stressors and rapid environmental change. We developed ten polymorphic microsatellite loci for *G. ventalina* to study the population genetics of this ecologically important coral throughout its range in the Caribbean.

METHODS & RESULTS

Tissue from *G. ventalina* colonies (4 cm x 4 cm, n=2) was collected at the Mote Tropical Research Laboratory, Summerland Key, FL, shipped to Cornell University live, and immediately ground in liquid nitrogen. Pulverized tissue was resuspended in 0.2 μm-filtered artificial seawater, and passed through a 70 μm filter to remove skeletal debris. The resulting filtered homogenate contained substantial amounts of calcium carbonate sclerites (skeletal elements) and dinoflagellate symbionts of the coral. *G. ventalina* tissue was separated from these contaminants via differential centrifugation through an 0.8:0.2 Percoll gradient (Stochaj and Grossman 1997) and lyophilized. Whole genomic DNA was extracted from the lyophilized tissue using a DNeasy Animal Tissue kit (Qiagen) according to the manufacturer's specifications. Genomic DNA was enriched for microsatellite repeats using a method adapted from Hamilton et al. (1999). In brief, whole genomic extracts were digested with *BsaA I* and *Hinc II* (New England Biolabs), ligated to a double-stranded SNX linker using T4 DNA ligase, and the pool of genomic fragments was enriched by hybridization with synthetic single-stranded biotinylated oligonucleotides (15-30 nt) of all possible di-, tri-, and tetranucleotide repeat motifs. Fragments were captured magnetically using Strepavidin-coated beads (New England Biolabs), and were made double-stranded using polymerase chain reaction (PCR) with the SNX primer. PCR products were digested with *Nhe I* and *Xba I* (New England Biolabs), ligated into a pUC 19 cloning vector and transformed into ElectroMAX DH5α-E Cells (Invitrogen). Transformed cells were selected for ampicillin resistance, and positive colonies were

transferred to nylon membranes and screened using a ^{33}P-labelled probe of pooled oligonucleotides of all possible di-, tri-, and tetranucleotide microsatellite motifs. Approximately 400 positively hybridized clones were sequenced using M13 forward primers and the BigDye Terminator Cycle Sequencing Kit (PerkinElmer) on an ABI Prism 377 DNA sequencer (Applied Biosystems). After trimming the pUC19 vector and SNX linker, all sequences containing microsatellite regions were aligned using the program SEQUENCHER v4.7 (Gene Codes Corporation) to identify duplicates. Primers were designed for 85 unique loci using PRIMERSELECT software (DNASTAR, Inc.). All primer pairs were initially tested on genomic extracts from 16 sea fan colonies from eight geographically distant localities (Florida Keys, Mexican Yucatan, Belize, Panama, Curacao, Barbados, Puerto Rico, & Bermuda). To verify that amplified loci belonged to the *G. ventalina* genome, and not to its dinoflagellate symbiont, all primers were also screened against genomic DNA extracts from 14 pure cultures of *Symbiodinium* B1/184, four of which were originally isolated from *G. ventalina*. Ten primer pairs amplified product from all or most *G. ventlina* colonies, but failed to amplify product from any of the *Symbiodinium* cultures, and were therefore considered to belong to the *G. ventalina* genome.

PCR screening was performed on a DNA Engine® (PTC-200™) Peltier Thermal Cycler (MJ Research) with the following protocol: 35 cycles of 95°C for 1 minute, a specified annealing temperature (Table 1.1) for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR amplification reactions were 10μL total volume and contained 10 ng of extracted DNA, 1μL 10x PCR reaction buffer (Invitrogen), 1.5mM $MgCl_2$, 0.2 μM dNTPs, 0.25U *Taq* polymerase (Invitrogen) and 40 pmol each of fluorescently-labeled forward primer (Applied Biosystems; Table 1.1) and unlabelled reverse primer. Fragments were analyzed on an ABI 3100 Automated

Table 1.1: Primer sequences, amplification conditions, and diversity for ten microsatellite loci in *Gorgonia ventalina*

Locus (GenBank)	Primer Sequence 5'-3'	Repeat Motif	T_a (°C)	N/N_a	Size Range (bp)	H_o	H_e
GV2_101	F: PET-CGGCGATCGTCGAAAAGAAGAC	$(TTTG)_{10}$ 8bp $(TTTG)_{13}$	65	229/52	394-572	0.93	0.96
(FJ390046)	R: AGGACCCCTCGATGCTGGAAGAGC						
GV2_65	F: PET-GGAATTCGGGTGCCTCAGGAT	$(GTT)_7$	65	237/7	148-165	0.63	0.74
(FJ390044)	R: AGAACAGCGGCAGCAGAGTCAC						
GV2_82	F: VIC-GTTCTGATCCGGATACTTGGAGGAAT	$(TTGTG)_{15}$	51	226/48	317-589	0.92	0.94
(FJ390045)	R: CGCCCACCTTTTTATTGCACTTATC						
GV47	F: 6FAM-GACAGAAGGAAAGCCACGTATCACGACACAT	$(TTC)_4$ 6bp $(TTC)_7$	67	237/4	175-202	0.15	0.14
(FJ390048)	R: CTAAGATCAAAGCAGAAAAAGAGAAACGTAAAGAAG						
GV1_15	F: 6FAM-TGCCTAGTGCTTGCATTGACATAAA	$(TG)_{11}$	65	237/11	222-239	0.59	0.65
(FJ390043)	R: CGGTGCGTGGGAACAAGATTAG						
GV31	F: PET-ATGCCTTGAACAAATTGAAAATGCAACTATTCTAC	$(CAT)_9$	67	231/8	235-268	0.44	0.56
(FJ390047)	R: TAAACTGATACTGCCACCGCCTCCTGATA						
GVc22	F: 6FAM-CCTGCTTTCCGCGTATGGGTTAGTTG	$(CT)_{14}$	67	235/15	155-199	0.64	0.75
(FJ390051)	R: CATGAAGTGAATTGGGAAGTCGCTGTAGG						
GVc3	F: NED-WATGAAAACGTGAAGGAAAAATGG	$(GT)_{10}$	66	237/23	117-159	0.74	0.77
(FJ390049)	R: GGTAGTCTGAAGATTGTATAGCAACATC						
GVc9	F: VIC-CACACTAACAGACGACGCAATTATTTCAACTCAAT	$(CT)_{12}$	67	231/21	147-287	0.63	0.70
(FJ390050)	R: GACCGGGCCTTTACGAAATCGTTTTAACATACTAA						
SYM203*	F: NED-GCTCCCTGGTACTTGAACGGCACGCTGAAAAT	$(AAC)_{25}$ 35bp $(AAAC)_{16}$	68	237/21	419-513	0.14	0.87
(FJ390052)	R: ACTGCGGAAAGCACATCTAGCATGGGACAACTGTAT						

Note. F, forward primer; R, reverse primer; T_a, annealing temperature; N, number of individuals scored; N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity.

* Locus SYM203 deviates significantly from Hardy-Weinberg equilibrium

Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GeneMapper v3.5 (Applied Biosystems) and verified by eye.

To quantify variation in the ten microsatellite loci, primers were tested on genomic extracts from 237 *G. ventalina* colonies collected at four sites along the Florida Keys Reef Tract (Carysfort Reef n=74; Conch Reef n=39; Alligator Reef n=56; Tennessee Reef, n=68). All ten primer pairs amplified polymorphic products, with the number of alleles per locus ranging from 4 to 52 (Table 1.1). Null alleles were rare with low frequencies occurring at five of the loci (GV2_101, 3.4%; GV2_82, 4.6%; GV31, 2.5%; GVc22, 0.8%; GVc9, 2.5%). Tests of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were performed using the program GENPOP ON THE WEB v3.4 (Raymond and Rousset 1995) using a Markov chain method with 10000 dememorization steps, 1000 batches of 10000 iterations, and a Bonferroni correction for multiple comparisons. All loci were in linkage equilibrium both within and across all sampling localities. Expected heterozygosity (Nei 1987) ranged from 0.14 to 0.96, and nine of the ten loci were in HWE within all localities. One locus, SYM203, showed a remarkable deficiency of heterozygotes within the four localities sampled (H_0=0.14 versus H_e=0.87). This departure from Hardy-Weinberg equilibrium is unlikely due to null alleles, since complete amplification failure was not observed in any of the 237 samples surveyed. Alternatively, the preponderance of apparent homozygotes at SYM203 would be expected if that locus belonged to the genome of *G. ventalina*'s dinoflagellate symbiont, though it was not amplified from any of the 14 *Symbiodinium* cultures tested.

These preliminary results suggest that these loci will provide a useful tool for population genetic studies of this important Caribbean coral. In combination with other recently developed microsatellite markers for *G. ventalina*'s dinoflagellate

symbiont (Andras *et al*., this issue) and fungal pathogen (Rypien and Andras 2008), these markers present an opportunity to examine patterns of population genetics in a complex suite of interacting organisms.

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CHAPTER 2:

Isolation and characterization of microsatellite loci in *Symbiodinium* B1/B184, the dinoflagellate symbiont of the Caribbean sea fan coral, *Gorgonia ventalina*

ABSTRACT

Here we report primers targeting ten microsatellite loci of dinoflagellates in the genus *Symbiodinium* (clade B1/B184) symbiotic with the Caribbean sea fan coral, *Gorgonia ventalina*. Primers were tested on 12 *Symbiodinium* B1/B184 cultures, as well as 40 genomic DNA extracts of *G. ventalina* tissue samples. All loci were polymorphic with allelic richness ranging from 4-16. Gene diversity ranged from 0.15 to 0.91. These primers provide powerful tools for examining the fine-scale population structure and dynamics of *Symbiodinium* within a single host species.

INTRODUCTION

Dinoflagellates of the genus *Symbiodinium* form endosymbioses with a wide variety of marine invertebrates (Rowan and Powers 1991a, Trench 1992, Rowan 1998, LaJeunesse 2001). Once thought to be a monotypic genus (Yonge 1973, Taylor 1974), *Symbiodinium* is now known to be genetically diverse (Coffroth and Santos 2005), and the phylogeny of the group has been well resolved (Takabayashi et al. 2004, Pochon et al. 2006). Moreover, numerous studies have demonstrated that *Symbiodinium* strains exhibit patterns of geographical and ecological zonation (Iglesias-Prieto et al. 2004, LaJeunesse et al. 2004a, Frade et al. 2008) and host specificity (LaJeunesse et al. 2004b). However, few studies have described the distribution of fine-scale genetic diversity within *Symbiodinium* lineages (Santos et al. 2003, Magalon et al. 2006). Thus, the population structure and dynamics of *Symbiodinium* associated with specific host species remain largely unknown.

The common sea fan coral, *Gorgonia ventalina*, is a conspicuous and abundant member of most Caribbean reef communities and associates exclusively with *Symbiodinium* type B1/B184 (LaJeunesse 2001, Kirk et al. 2005, Van Oppen et al. 2005), the most prevalent lineage in the Caribbean Sea (LaJeunesse 2002). This specificity makes this system ideal to study the fine-scale population structure of an ecologically important *Symbiodinium* strain within a single host. We developed ten polymorphic microsatellite loci for *Symbiodinium* B1/B184 isolated from *G. ventalina* to investigate the population genetics of these symbionts.

METHODS & RESULTS

Tissue explants from *G. ventalina* colonies were collected at Mote Marine Lab, Summerland Key, FL (n=2, 4cm x 4cm) and Sandy Point, Bahamas (n=4, 4cm x 4cm), shipped to Cornell University live, and immediately ground in liquid nitrogen. Pulverized tissue was resuspended in 0.2 μm filtered artificial seawater and passed through a 70 μm filter to remove skeletal debris. *Symbiodinium* cells were isolated from the crude homogenate via differential centrifugation through an 0.8:0.2 Percoll gradient (Stochaj and Grossman 1997) and the algal layer was rinsed twice with isolation buffer (Rowan and Powers 1991b). Whole genomic DNA was extracted from purified *Symbiodinium* cells using the DNeasy Animal Tissue kit (Qiagen) and enriched for microsatellite repeats using a method adapted from Hamilton *et al*. (1999). Genomic extracts were digested with *BsaA I* and *Hinc II* (New England Biolabs), ligated to a SNX linker using T4 DNA ligase, and enriched by hybridization with synthetic single-stranded biotinylated oligonucleotides (15-30 nt) of all possible di-, tri-, and tetranucleotide repeat motifs. Fragments were captured magnetically using Strepavidin-coated beads (New England Biolabs), and were made double-stranded using polymerase chain reaction (PCR) with the SNX primer. PCR products

were digested with *Nhe I* and *Xba I* (New England Biolabs), ligated into a pUC 19 cloning vector and transformed into ElectroMAX DH5α-E Cells (Invitrogen). Transformed cells were selected for ampicillin resistance, and positive colonies were transferred to nylon membranes and screened using a ^{33}P-labelled probe of pooled oligonucleotides of all possible di-, tri-, and tetranucleotide microsatellite motifs. Approximately 200 positively hybridized clones were sequenced using M13 forward primer and the BigDye Terminator Cycle Sequencing Kit (PerkinElmer) on an ABI Prism 377 DNA sequencer (Applied Biosystems). Primers were designed for 60 unique loci using the program PRIMERSELECT v7.2.1(DNASTAR, Inc.), 10 of which consistently amplified polymorphic products.

PCR screening was performed on a DNA Engine® (PTC-200™) Peltier Thermal Cycler (MJ Research) with the following protocol: 35 cycles of 95°C for 1 minute, a specified annealing temperature (Table 2.1) for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR amplification reactions were 10μL total volume: 10 ng template DNA (except when otherwise noted below), 10mM Tris-HCl (pH 8.3), 1.5mM $MgCl_2$, 0.2 μM dNTPs, 0.25U *Taq* polymerase (Invitrogen) and 0.2 μM each of fluorescently-labeled forward primer (Applied Biosystems; Table 2.1) and unlabelled reverse primer. Fragments were analyzed on an ABI 3100 Automated Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEMAPPER v3.5 (Applied Biosystems) and validated by eye.

To verify that primers amplified targets specific to the algal symbiont and not the coral host, they were screened with monoclonal *Symbiodinium* B1/B184 cultures isolated from seven different Cnidarian host species (*Gorgonia ventalina*, *Pseudopterogorgia elisabethae*, *Plexaura kuna*, *Pocillopora damicornis*, *Aiptasia pulchella*, *Aiptasia pallida*, and *Zoanthus pacificus*). To determine the lower

Table 2.1: Primer sequences, amplification conditions, and diversity for ten microsatellite loci in *Symbiodinium sp.* B1/B184

Locus (GenBank)	Primer sequence (5'-3')	Repeat sequence	T_a	N/N_a	Size (bp)	Gene divesity	Lower template threshold (ng/#cells)
SYM155	F: 6FAM-GTCCCCTTCTTCTGCTTACCTGTTTTAGGAGA	$(CAA)_5$ 5bp $(CAA)_8$ TAA $(CAA)_{10}$	54	178/12	222-277	0.81	0.05/~25
(FJ390041)	R: AGGACCCCTCGATGCTGGAAGAGC						
GV1C	F: 6FAM-TCTAGGGGCCATGGATAAAACTACCA	$(AAC)_8$	56	178/13	279-327	0.89	0.025/~13
(FJ390033)	R: CGCATACGCATGATTCGATACTGAG						
SYM254	F: VIC-TGAAAAGTGTCGAAGTAAGTACCCAAAAAGAAGAAC	$(CAA)_{18}$	54	178/16	136-189	0.91	0.025/~13
(FJ390042)	R: TGTGCGGCAGTGAACCCATCGTG						
GV28	F: PET-AGGATTCACAAAGACCGTCTCACCTCT	$(TA)_7$	56	178/7	253-264	0.58	0.05/~25
(FJ390037)	R: TGGATAATTATACCAGCAGCTTGTGGGTTGC						
GV2_100	F: VIC-TATCAAGGTCCTATTTTCACAGCACAA	$(GTT)_3$ GAT $(GTT)_{24}$ 11bp $(GTT)_{19}$	54	178/13	252-282	0.87	0.025/~13
(FJ390036)	R: ACAGGCGAGGTATAGTATTGAGTAAAAGAA						
GV2_70	F: 6FAM-CTTGGGCACTAATTTCTGTTTTTGTCC	$(GTT\ GCT)_8$ $(GTT)_{24}$	56	178/14	367-411	0.81	0.025/~13
(FJ390035)	R: TCATGCCGCTATGCCCTTGTAATA						
SYM7	F: PET-ACAGATAATGGATCAACGCCTCTTTACATAGCAG	$(TGT)_6$ CGT $(GGT)_5$	63	178/4	462-477	0.15	1.0/~500
(FJ390040)	R: AATAAAAAGGCGGAACCAATTACTGACACTAAGC						
GV2	F: PET-TGTAGATGAGGTGCGGTCAG	$(CT)_{12}$	52	178/8	160-202	0.82	0.05/~25
(FJ390034)	R: ACAATGATAGGGGGCTCACA						
GV42	F: 6FAM-ACTTCCTTTGTTGTGCGTCA	$(GA)_{13}$	54	178/6	153-165	0.68	0.005/~3
(FJ390038)	R: GGCGGGTACTTTTTGAAGGT						
GV44 (FJ390039)	F: PET-AATCGTTTTTGCGTGGTCTC R: TCAATTCGTACCCGACAATG	$(CT)_{10}$ $(GT)_2$ $(CT)_{13}$ $(GT)_2$	54	178/13	116-175	0.84	0.1/~50

Note. F, forward primer; R, reverse primer; T_a, annealing temperature; N, number of individuals scored; N_a, number of alleles.

threshold of amplification, separate PCR reactions were conducted using serial dilutions of template DNA (0.5, 0.2, 0.1, 0.05, 0.025, & 0.005 ng per 10 μl PCR reaction). To test for allelic dropout with mixed DNA templates, primers were challenged with mixtures of genomic DNA from separate *Symbiodinium* B1/B184 cultures with distinct genotypes. A three-template mixture was tested at five different mixture ratios (1:1:1, 2:1:1, 10:1:1, 100:1:1, 1000:1:1) while maintaining the total template DNA at 10 ng. A four-template mixture of equimolar proportions also was tested. To quantify naturally occurring variation in the ten microsatellite loci, primers were screened against DNA extracted from 178 *G. ventalina* colonies collected at four sites along the Mesoamerican Barrier Reef in Mexico (Isla Mujeres n=35; Akumal n=55; Cozumel n=51; Tulum n=37).

All ten primer pairs amplified a single product in each of the seven *Symbiodinium* cultures tested, consistent with previous observations that *Symbiodinium* is haploid in its vegetative state (Santos and Coffroth 2003). The lower amplification threshold of template DNA for each locus ranged from 0.005 ng to 1.0 ng (Table 2.1). Based on an average *Symbiodinium* B1 genome size of two pg (LaJeunesse et al. 2005), detection thresholds were estimated to range from as few as 3-500 individual cells. In mixed-template PCRs, all primer pairs were capable of amplifying four distinct templates (the maximum number tested) at equimolar concentrations. Most primer pairs were capable of reliably amplifying two low-frequency templates in combination with a third more concentrated template at a mixture ratio of 10:1:1, but were unable to amplify these same low-frequency templates at relative dilutions of 100:1:1 or 1000:1:1, suggesting that template competition significantly reduces amplification thresholds of loci in mixed-template reactions.

All ten primer pairs amplified polymorphic products from all 178 *G. ventalina* samples, with allelic richness ranging from 4-16 (Table 2.1). The majority of *G. ventalina* colonies screened had a single allele per locus, however 14 colonies (7.8%) yielded multiple alleles for at least one locus. As *Symbiodinium* are haploid, and amplification of monoclonal *Symbiodinium* cultures produced only one allele per locus, we interpret the occurrence of multiple alleles per locus in *G. ventalina* tissue as evidence of multiple strains of *Symbiodinium* within a single host colony.

As *Symbiodinium* is haploid, heterozygosity is not defined, and Hardy-Weinberg equilibrium could not be determined. Gene diversity (Nei 1987) was calculated using FSTAT (Goudet 1995) with values ranging from 0.15-0.91 (Table 2.1). Tests for linkage disequilibrium were performed using the program GENEPOP ON THE WEB (Raymond and Rousset 1995), using a Markov chain method with 10000 dememorization steps, 1000 batches of 10000 iterations, and a Bonferroni correction for multiple comparisons. All pairs of loci showed significant departure from linkage equilibrium in global tests and within at least one sampling locality. Linkage disequilibrium across and within localities is not surprising as *Symbiodinium* reproduces asexually *in hospite* (Freudenthal 1962, Fitt and Trench 1983), and recombination may be limited. However, each pair of loci was in linkage equilibrium in at least one locality, suggesting the observed linkage disequilibrium is not due to the physical association of loci.

The primers reported here consistently amplify polymorphic microsatellite loci from both *Symbiodinium* cell cultures isolated from distantly-related Cnidarian hosts and DNA extracts of the intact *G.ventalina*/*Symbiodinium* holobiont, suggesting broad applicability. Furthermore, all primer pairs were able to detect multiple *Symbiodinium* strains within a single host. Microsatellite primers have also been reported for the host, *G. ventalina* (Andras *et al*., this issue) and *Aspergillus sydowii*, a fungal

pathogen of *G. ventalina* (Rypien and Andras 2008). Collectively, these markers provide a powerful tool for the study of this coral host/symbiont/pathogen system.

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CHAPTER 3:

Range-wide population genetic structure of *Symbiodinium* associated with the Caribbean sea fan coral, *Gorgonia ventalina*

ABSTRACT

Numerous marine invertebrates form endosymbiotic relationships with dinoflagellates of the genus *Symbiodinium*. However, few studies have examined the fine-scale population structure of these symbionts. Here, we elucidate the genetic structure of *Symbiodinium* type B1/B184, the most prevalent symbiont lineage in the Caribbean, throughout the entire range of its host coral, *Gorgonia ventalina*. We used ten polymorphic microsatellite loci to survey 35 localities spanning 3124 km across the Caribbean and Western Atlantic. Diversity of *Symbiodinium* haplotypes was generally low within colonies of *G. ventalina*, but high among colonies. Despite high haplotypic diversity, significant evidence of clonal reproduction in *Symbiodinium* was detected, and most clones were distributed within localities, not among them. Pairwise measures of F_{st} and R_{st} showed significant differentiation in 99% of possible comparisons between localities, suggesting low levels of gene flow in these dinoflagellates. Genetic clustering analyses identified six genetic clusters whose cohesive distribution delimited four broad biogeographic regions. There was evidence of some connectivity among regions, corresponding with known geographic and hydrodynamic features. However, estimates of recent migration rates show that most regions are almost entirely self-recruiting, indicating that populations of this strain of *Symbiodinium* are demographically independent at the regional scale. Fine-scale spatial surveys of *G. ventalina* colonies failed to detect differentiation among *Symbiodinium* at the scale of meters. However, significant differentiation was observed among *Symbiodinium* hosted by co-occurring *G. ventalina* colonies of

different size/age classes. This cohort effect suggests that *Symbiodinium* may have an epidemic population structure, whereby *G. ventalina* recruits are infected by the locally predominant symbiont strain(s), which change over time.

INTRODUCTION

Most, if not all, metazoan species exist in symbiotic relationships with microbes (Moran 2007), and the population structure of microbial symbionts is of central relevance to the ecology and evolution of their symbioses. Most directly, the environmental availability of symbionts may impose limits on the distribution of the host, particularly in cases where the association is obligate. Additionally, heritable phenotypic variation among symbiont populations may expand the functional diversity of the intact symbiotic association (holobiont). The population structure of symbionts may also affect the evolutionary trajectory of symbiosis along the continuum from parasitism to mutualism. For example, it has been hypothesized that limited dispersal and local population structure of symbionts may be important characteristics for the evolution and maintenance of mutualism, whereas high symbiont dispersal can impede or disturb host/symbiont coadaptation and promote parasitism (Frank 1994, Bever and Simms 2000).

Despite the importance of the subject, relatively few studies have investigated the population structure of microbial symbionts, especially in marine systems. However, one important group of marine microbial symbionts whose population structure has recently begun to receive attention is *Symbiodinium*, a genus of photosynthetic dinoflagellates. These unicellular algae form symbioses with a broad range of hosts from a number of phyla including Cnidaria, Porifera, Mollusca, Platyhelminthes, Foraminifera, and Ciliophora (Trench 1979, Carlos et al. 1999, Pawlowski et al. 2001, Lobban et al. 2002). However, the most conspicuous and

diverse *Symbiodinium* associations are those formed with Cnidarians of the class Anthozoa, particularly shallow-water tropical corals. In corals, *Symbiodinium* reside within gastrodermal cells, where they translocate as much as 95% of their photosynthetic product to the host in the form of glycerol, carbohydrates, and peptides (Muscatine 1967, Muscatine et al. 1984). Additionally, the active uptake of carbon dioxide by *Symbiodinium* helps to maintain the alkaline environment necessary for the deposition of calcium carbonate skeletal elements by the host (Pearse and Muscatine 1971). In return, *Symbiodinium* benefit from the consistent source of ammonium and phosphate present in the coral's metabolic byproducts, as well as an ideal environment for collecting light for photosynthesis (Enriquez *et al.* 2005). This mutualism has allowed reef ecosystems to thrive and diversify in the oligotrophic waters of tropical oceans (Muscatine and Porter 1977), yet it is notoriously sensitive to a number of environmental factors, most notably temperature. Indeed, the dissolution of the coral/algal symbiosis, known as coral bleaching, is usually fatal for the coral (Glynn 1993), and the increasing frequency of bleaching due to anthropogenic ocean warming is responsible for the widespread deterioration and loss of reef ecosystems (Hoegh-Guldberg 1999, Hughes et al. 2003).

Due to a paucity of obvious distinguishing morphological characters, *Symbiodinium* was once thought to be a cosmopolitan, monotypic genus (Taylor 1974). However, molecular genetic data have since revealed high diversity and deep divergence commensurate with that found among distinct orders of free-living dinoflagellates (Rowan and Powers 1992). Phylogenies based on a variety of ribosomal, mitochondrial and chloroplast sequences have congruently resolved eight broad clades, A-H (reviewed in Baker 2003, Coffroth and Santos 2005), each of which contains a diversity of more closely-related phylotypes. A number of these phylotypes have been found to exhibit distinct geographic distribution (Baker 2003, LaJeunesse et

al. 2004a), host specificity (LaJeunesse *et al.* 2004b), depth zonation (Rowan and Knowlton 1995), and physiology (Iglesias-Prieto and Trench 1997, Robison and Warner 2006, Warner et al. 2006), leading some authors (LaJeunesse 2001, LaJeunesse 2005, Sampayo et al. 2009) to propose that these groups represent distinct species.

Molecular studies at the clade and phylotype level have substantially advanced our knowledge of the diversity, distribution, and ecology of *Symbiodinium*, and have expanded our notion of the basic unit of coral biodiversity (Rowan and Knowlton 1995). More recently, attention has turned to the fine-scale genetic diversity residing within *Symbiodinium* phylotypes. These few studies have found that, unlike putative species-level phylotypes, which can exhibit circum-global distributions, population-level diversity is often highly structured over relatively small spatial scales. For example, Santos *et al*. (2003b), using two polymorphic microsatellites, found that populations of *Symbiodinium* phylotype B1/184 (sensu LaJeunesse 2001, Santos et al. 2003a) hosted by the gorgonian coral, *Pseudopterogorgia elisabethae*, were differentiated across tens of kilometers in the Bahamas. Similarly, Howells *et al*. (2009), using four polymorphic microsatellites, found significant differentiation in populations of *Symbiodinium* phylotype C1 hosted by the alcyonacean coral, *Sinularia flexibilis*, separated by as little as 16 km on the Great Barrier Reef. A study of *Symbiodinium* type B1/184 symbiotic with Caribbean scleractinian corals of the genus *Montastrea* found that most haplotypes in the Bahamas and the Florida Keys were endemic to a single reef (Thornhill *et al.* 2009). Taken together, these results indicate that substantial genetic diversity does commonly exist within *Symbiodinium* phylotypes, and that this diversity may be highly structured across small scales among and within local reefs.

Such detailed studies of the fine-scale genetic diversity within *Symbiodinium* lineages have the potential to identify source and sink populations, describe patterns of connectivity, quantify gene flow, and elucidate mechanisms of dispersal. This information will, in turn, enhance our understanding of the biology of the coral/algal holobiont and shed light on the little known ecology of *Symbiodinium* in its free-living state. The structure and dynamics of *Symbiodinium* populations directly affect the ability of corals to recover their vital symbionts after bleaching episodes. Moreover, if population-level genetic diversity in *Symbiodinium* correlates with significant functional diversity, this may provide long-lived corals an added dimension of flexibility to "adapt" to environmental change. An accurate understanding of these phenomena will become increasingly relevant for the effective conservation and management of reef ecosystems in the face of escalating anthropogenic threats such as ocean warming.

We describe here the population structure of *Symbiodinium* B1/184 associated with the sea fan coral, *Gorgonia ventalina*, a conspicuous and abundant inhabitant of reefs throughout the Caribbean (Bayer 1961, Opresko 1973, Yoshioka and Yoshioka 1989, Sanchez et al. 1997). *G. ventalina* is only known to associate with a single phylotype of *Symbiodinium*, type B1/184, throughout its range (Goulet and Coffroth 2004, Kirk et al. 2005, Van Oppen et al. 2005, Kirk et al. 2009), and thus provides an ideal system to investigate the population structure of a *Symbiodinium* phylotype within a single host. Kirk *et al.* (2009) recently examined the population structure of *Symbiodinium* from *G. ventalina* in the Florida Keys, using six microsatellite loci and found highly local population structure on the scale of tens of kilometers. To examine the generality of these results, and to investigate emergent patterns at both broader and finer spatial scales, we expand upon the study of Kirk *et al.* (2009), using ten polymorphic microsatellite loci (Andras *et al.* 2009) to describe the population

genetics of *Symbiodinium* hosted by *G. ventalina* from 35 localities spanning more than 3100 km across the entire host range in the Caribbean and Western Atlantic. We hypothesize that the pattern of strong local population structure observed in previous regional studies is a result of the basic dispersal limits of *Symbiodinium* and, therefore, would be consistent across the entire range. However, we also expect that occasional long-distance dispersal via ocean currents does occur and would leave its signature in patterns of inter-regional connectivity.

METHODS

Population Sampling

A total of 1624 samples were collected from 35 sites spanning the range of *G. ventalina* in the Caribbean and Western Atlantic (Figure 3.1, Table 3.1). Sites ranged in depth from 5-24 m, with most around 7 m. Where possible, sites were chosen for consistency in habitat characteristics, namely, hard-bottom fore-reef sites dominated by gorgonian communities. At each site, adult colonies of *G. ventalina* (>30 cm height) were haphazardly sampled along several swimming transects across an area measuring approximately 25 m x 25 m. From each sampled colony, a 4 cm x 4 cm tissue explant was cut from the apical colony edge and transferred to a labeled bag. The number of individuals sampled per site (Table 3.1) varied based on availability, with an average of 46. After collection, samples were transferred to individual 2.0 ml plastic tubes containing a 20% salt-saturated dimethyl sulfoxide (DMSO) solution for preservation (Seutin et al. 1991, Dawson et al. 1998).

To resolve the population structure of *Symbiodinium* at a finer scale, two additional sets of *G. ventalina* tissue samples were collected from Pickles Reef in the Florida Keys. First, to investigate variation of *Symbiodinium* within individual *G. ventalina* colonies, tissue samples were collected at five positions (left edge, top edge,

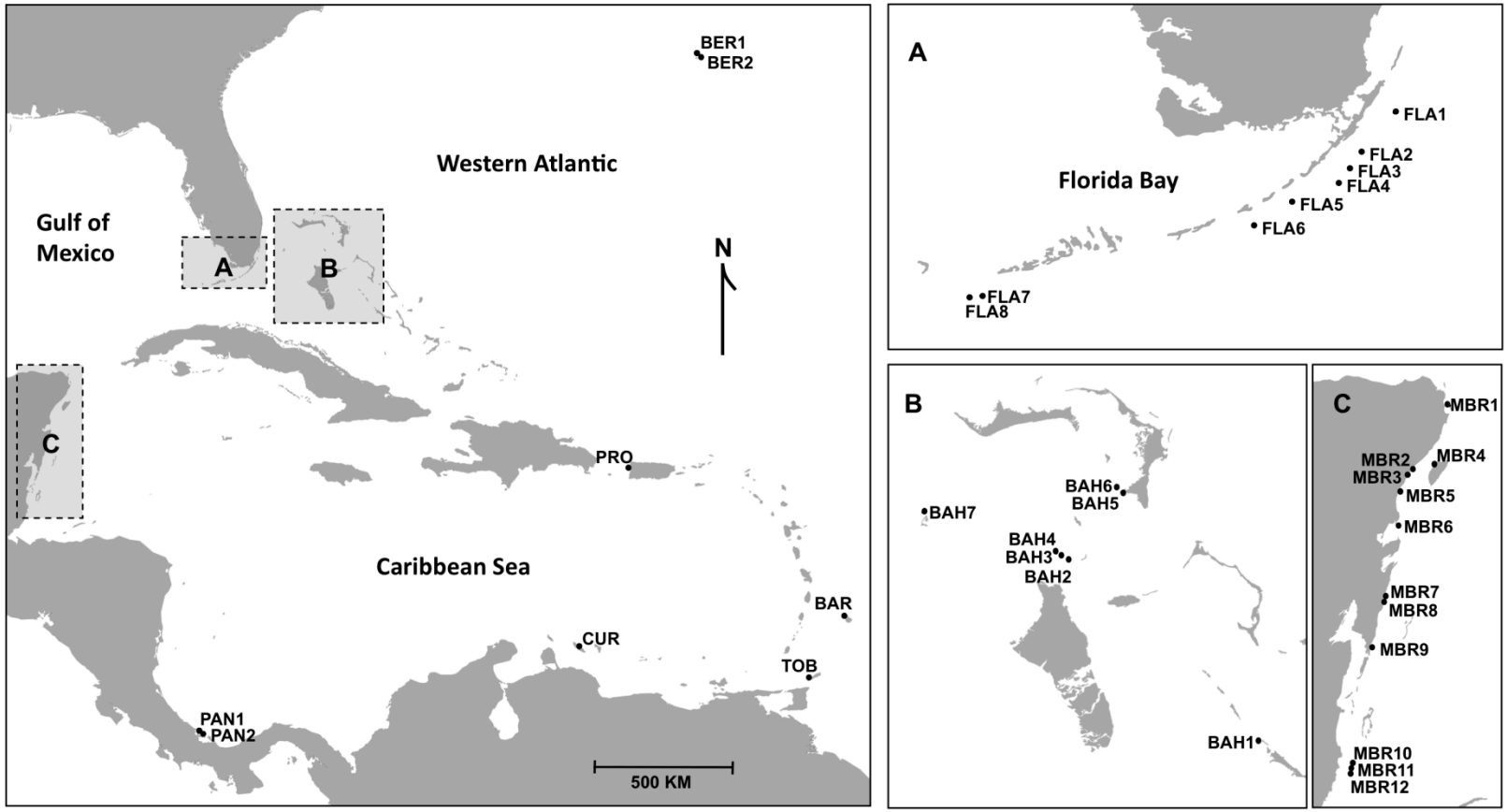

Figure 3.1: Map of 35 localities across the Caribbean Sea and Western Atlantic where *Gorgonia ventalina* colonies were sampled for this study. Regional maps of the Florida Keys (**A**), Bahamas (**B**), and Mesoamerican Barrier Reef (**C**) are enlarged.

Table 3.1: Name, abbreviation, depth, GPS coordinates, collection date, and percentage of clonal haplotypes for all 35 sample locations. Localities are grouped by geographic region.

Region	Site	Site Code	Depth(m)	GPS	Date Collected	n	% Clonal
Florida Keys	Florida, Carysfort	FLA1	5	N 25.22°, W 80.21°	8/03	90	25.7%
	Florida, Molasses	FLA2	6	N 25.00°, W 80.37°	8/03	68	30.4%
	Florida, Pickles	FLA3	6	N 24.98°, W 80.41°	8/03	75	22.6%
	Florida, Conch	FLA4	8	N 24.95°, W 80.45°	8/03	47	41.0%
	Florida, Alligator	FLA5	5	N 24.84°, W 80.62°	8/03	68	33.9%
	Florida, Tennessee	FLA6	6	N 24.74°, W 80.78°	8/03	83	29.4%
	Florida, Western Dry Rocks	FLA7	6	N 24.44°, W 81.92°	8/03	35	24.1%
	Florida, Sand Key	FLA8	5	N 24.45°, W 81.87°	8/03	33	44.4%
Mesoamerican Barrier Reef	Mexico, Isla Mujeres, Tavos	MBR1	9	N 21.24°, W 86.73°	4/03	47	35.9%
	Mexico, Akumal, Media Luna	MBR2	9	N 20.40°, W 87.30°	4/03	68	21.4%
	Mexico, Akumal, South Point	MBR3	9	N 20.38°, W 87.31°	4/03	12	30.0%
	Mexico, Cozumel, Paradise	MBR4	8	N 20.51°, W 86.94°	4/03	69	52.6%
	Mexico, Tulum, La Piscina	MBR5	12	N 20.22°, W 87.74°	5/03	52	18.6%
	Mexico, Punta Allen	MBR6	9-12	N 19.78°, W 87.43°	7/06	36	43.3%
	Mexico, Mahahual, El Jardin	MBR7	17	N 18.85°, W 87.63°	5/03	42	42.9%
	Mexico, Mahahual, Site 2	MBR8	8	N 18.84°, W 87.64°	5/03	53	50.0%
	Mexico, Xcalak, Dona Nica	MBR9	11	N 18.29°, W 87.81°	5/03	50	26.8%
	Belize, Southwater Caye, Long Reef	MBR10	5	N 16.77°, W 88.08°	5/03	51	47.6%
	Belize, Carrie Bow Caye	MBR11	5	N 16.80°, W 88.08°	5/03	24	45.0%
	Belize, Southwater Caye, East Side	MBR12	9	N 16.81°, W 88.07°	5/03	51	35.7%
Panama	Panama, Bocas del Toro, Punta Vieja	PAN1	5-8	N 9.260°, W 82.12°	10/05	34	32.1%
	Panama, Bocas del Toro, Hospital Point	PAN2	5-8	N 9.333°, W 82.21°	10/05	20	47.1%
Curacao	Curacao	CUR	9-12	N 12.11°, W 68.97°	7/06	58	33.3%
Tobago	Tobago	TOB	9-12	N 11.19°, W 60.79°	4/08	53	34.1%
Barbados	Barbados	BAR	9-12	N 13.17°, W 59.64°	9/06	74	23.0%
Puerto Rico	Puerto Rico, Steps Beach, Rincon	PRO	5-6	N 18.35°, W 67.26°	8/06	62	27.5%
Bahamas	Bahamas, Lee Stocking Island	BAH1	5-6	N 23.78°, W 76.13°	5/03	33	37.0%
	Bahamas, Chub Cay	BAH2	9-12	N 25.40°, W 77.92°	5/05	25	33.3%
	Bahamas, GPS121	BAH3	11-12	N 25.42°, W 77.98°	5/04	19	37.5%
	Bahamas, Rum Cay	BAH4	12-15	N 25.45°, W 78.03°	5/04	12	50.0%
	Bahamas, Sandy Point	BAH5	8-9	N 25.99°, W 77.42°	5/04	25	42.9%
	Bahamas, Gorda Rock	BAH6	5-6	N 26.04°, W 77.47°	5/04	22	38.9%
	Bahamas, Bimini	BAH7	21-24	N 25.81°, W 79.28°	5/05	17	85.7%
Bermuda	Bermuda, Castle Harbour	BER1	5-6	N 32.33°, W 64.67°	5/07	55	26.7%
	Bermuda, Crescent	BER2	5-6	N 32.39°, W 64.79°	5/07	61	22.0%

right edge, center, base) from twenty large colonies (>30 cm height). Second, to investigate population structure of *Symbiodinium* hosted by different size/age classes of *G. ventalina* within a reef, we sampled all large (≥30 cm) and small (≤10 cm) colonies from two 1 m^2 plots separated by ~14 m on a continuous tract of hard-bottom reef. We chose these size classes, as they have been identified as demographically non-overlapping based on an estimated age-height relationship of *G. ventalina* (Stephen Ellner, personal communication). This subset allowed us to assess the relative contributions of host-colony age and spatial proximity in determining relatedness among *Symbiodinium*.

DNA Extraction, Amplification, and Genotyping

Whole genomic DNA was extracted from 1cm x 1cm tissue subsamples using DNeasy Animal Tissue Kits (Qiagen). Extracted DNA was quantified using a Nanodrop spectrophotometer and diluted to 10 ng/μl with reagent-grade water (Sigma). Each sample was genotyped via polymerase chain reaction (PCR) using primers and run conditions for 10 previously reported microsatellite loci (Andras *et al.* 2009). PCR products were analyzed on an ABI 3100 Automated Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEMAPPER v3.5 (Applied Biosystems) and validated by eye.

Although *Symbiodinium* is haploid *in hospite* (Santos and Coffroth 2003), multiple alleles were detected at some loci in some samples. Since the microsatellite markers used in this study amplified only a single allele from a number of pure clonal cultures of *Symbiodinium* (Andras *et al.* 2009), we conclude that multiple alleles per sample were due to multiple strains of *Symbiodinium* within a single *G. ventalina* colony. In samples harboring multiple *Symbiodinium* strains, allelic phase could not

be determined and discrete multilocus haplotypes could not be constructed. To include these individuals in our analyses, we utilized two alternative datasets: a full dataset and a curtailed dataset. The full dataset included all samples (n=1640), with genetic data coded as dominant markers based on the presence or absence of alleles (as in Magalon *et al.* 2006). To determine whether alleles that co-occurred within a single *G. ventalina* colony were drawn from the same distribution as those that occurred individually, we conducted Fisher Exact Tests of genic differentiation using the program GENEPOP ON THE WEB (Raymond and Rousset 1995). For each locus at each sampling locality, all co-occurring alleles were pooled as one group and compared to the pool of alleles that occurred individually.

We also constructed a curtailed dataset of samples containing no more than one allele per locus (n=1341, 82% of the full dataset), with genetic data coded as codominant multilocus genotypes based on the number of microsatellite motif repeats per locus. While the curtailed dataset may represent a less comprehensive survey of allelic diversity than the full dataset, it provides greater analytical power, as patterns of linkage between markers can be assessed, and specific evolutionary models can be applied. Indices of diversity and population differentiation were calculated for both datasets and compared.

Genetic Diversity

To quantify genetic diversity for each locus at each sampling locality, we calculated Nei's diversity corrected for sample size (Nei 1987) using the program GENODIVE 2.0b12 (Meirmans and van Tienderen 2004). We also calculated rarefaction corrected allelic richness with all localities scaled to a common sample size of 10 genes, using the program HP-RARE 1.0 (Kalinowski 2005). Identical multilocus haplotypes (clonemates) were identified in the curtailed dataset using the

program GIMLET (Valiere 2002), and significance tests for clonal population structure were performed with GENODIVE. This analysis compares the observed diversity of multilocus haplotypes to that of permuted datasets, which approximate expectations under random sexual reproduction (Gomez and Carvalho 2000, Stenberg et al. 2003). A significantly lower observed diversity is interpreted as evidence of clonal population structure. Nei's corrected diversity index (Nei 1987) was used as the test statistic with 10000 permutations that randomized alleles within populations. Correlations between gene diversity and latitude were assessed based on a bivariate analysis of variance (ANOVA) implemented with the program JMP 7.0.

Population Structure

To investigate the partitioning of genetic diversity among localities and regions, we performed analyses of molecular variance (Excoffier *et al.* 1992) using the program ARLEQUIN v3.0 (Excoffier *et al.* 2005). Samples were grouped by collection locality nested within region. Regional designations (Table 3.1) were defined based on the geographic proximity of sampling localities and oceanographic barriers between them. Both the complete and curtailed datasets were analyzed assuming an infinite allele model (IAM; Kimura and Crow 1964). In addition, the curtailed dataset was analyzed based on an assumption of a stepwise mutation model (SMM; Ohta and Kimura 1973, Valdes et al. 1993). The SMM assumes that microsatellites mutate by slipped-strand mispairing during replication, resulting in the incremental gain or loss of single repeat motifs (Ellegren 2004). Consequently, the difference in length between two alleles is inversely proportional to their relatedness. Pairwise measures of differentiation between sampling localities were also calculated using ARLEQUIN. Estimates of F_{st} (Weir and Cockerham 1984), which assumes an

IAM, were calculated for both the complete and curtailed datasets, and R_{st} (Slatkin 1995), an F_{st} analog based on a SMM, was calculated for the curtailed dataset

We also inferred structure in our data using a Bayesian clustering approach implemented by the program STRUCTURE 2.2.3 (Pritchard et al. 2000, Falush et al. 2003, 2007). This method probabilistically assigns individuals membership among K populations (where K is specified by the user) based solely on genotypic data. Because STRUCTURE assigns cluster membership by identifying groupings of individuals that minimize within-group linkage disequilibrium (LD), alleles within sub-populations are assumed to be in linkage equilibrium. To verify that this assumption was met, we performed pairwise exact tests of gametic LD on the curtailed dataset using GENEPOP ON THE WEB (Raymond and Rousset 1995) with 10000 dememorization steps and1000 batches of 10000 iterations. We also ran a separate LD analysis after removing all repeated haplotypes from the dataset, as the incidence of clones can produce artificial patterns of non-random association between alleles that are not truly linked (Smith *et al.* 1993)

In STRUCTURE, we employed a model that allowed admixture, assumed correlated allele frequencies among populations, and did not incorporate population origin information. Each run consisted of $2x10^6$ Markov Chain Monte Carlo (MCMC) iterations after a burn-in of $2x10^6$ steps. This run-length was sufficient to achieve stabilization of the posterior probability of the data [lnP(D)]. Twenty independent runs were performed at each K from 1 through 36. The authors of STRUCTURE advise caution when estimating the number of populations, particularly for large complex datasets like ours where the posterior probability continues to increase with increasing K (Pritchard *et al.* 2000). In such cases, there is a risk of overestimating K beyond a biologically meaningful value. To guide our estimation of the number of populations in our dataset, we employed iterative rounds of the ΔK method (Evanno *et*

al. 2005) as described by Coulon *et al*. (2008). This approach identifies the number of clusters (K) at the highest level of population structure by determining the maximum second-order rate of change in posterior probability (ΔK) over a range of K. By calculating the maximum ΔK iteratively for successively parsed datasets, hierarchically structured metapopulations can be resolved into nested levels of differentiation. We applied this method repeatedly until each cluster could be divided no further (i.e. optimal K for each cluster = 1), or until the resulting clusters were not biologically sensible. Once the best number of populations was selected, cluster membership for the runs with the ten highest posterior probabilities were matched and averaged by the program CLUMPP (Jakobsson and Rosenberg 2007), using the Greedy algorithm with 100000 random input orders.

In addition to Bayesian clustering, we also constructed an unrooted dendrogram based on Nei's unbiased genetic distance (Nei 1978) to visualize relationships between localities. The dendrogram was generated with the unweighted pair group method with arithmetic mean (UPGMA) using the program TOOLS FOR POPULATION GENETICS ANALYSIS (TFPGA) v1.3 (Miller 1997). One hundred thousand random bootstrap permutations were performed across loci to generate confidence values for each node of the dendrogram.

Spatial Analyses

To evaluate the relationship between genetic differentiation and geographic distance, we performed Mantel tests (ARLEQUIN 3.0; 1000 random permutations) of genetic differentiation (F_{st} & R_{st}) versus Euclidian (straight-line geographic) distances among localities. We also performed tests of spatial genetic autocorrelation to evaluate whether the degree of genetic similarity between individuals is correlated with the geographic distance between them. Unlike Mantel tests, which identify strong

patterns of differentiation extending over the full geographic sample range, spatial autocorrelation analyses allow for the independent consideration of comparisons within different distance classes, thereby providing greater resolution of spatial genetic structure (Clark and Richardson 2002, Epperson 2005). We used the program GENALEX 6.1 (Peakall and Smouse 2006) to evaluate spatial autocorrelation in our dataset following two alternative approaches, one using *fixed distance classes* of the same size, and another using *multiple distance classes* of increasing size. All analyses were based on pairwise genetic distances among all individuals which were calculated as described by Peakall *et al.* (1995). For the *fixed distance class* analysis, we measured the autocorrelation coefficient of genetic distance (r) within geographic distance classes of a fixed size (50 km, 62 total distance classes). The behavior of r over increasing distance can illustrate the shifting balance between gene flow and genetic drift. In cases where positive spatial structure is present, r decreases with increasing distance size class, and the point at which r first becomes zero (i.e. the x-intercept of the autocorrelogram) provides an estimate of the approximate extent of positive spatial genetic structure (Sokal and Wartenberg 1983, Smouse and Peakall 1999, Peakall et al. 2003, Epperson 2005). Since the x-intercept of r has been shown to depend on the size of the fixed distance class employed (Peakall et al. 2003), we also implemented an alternative approach that is less sensitive to distance class size. In this *multiple distance class* approach, r was calculated for 32 geographic distance classes of increasing size, ranging from 100 km to 3200, the maximum distance between sampling localities. For all spatial autocorrelation analyses, significance was assessed relative to 1000 random permutations of the dataset, and 95% confidence intervals of r were generated from 1000 bootstrap replicates. Because localized clonal population structure has the potential to drive spatial patterns of genetic differentiation due to the co-occurrence of individuals with zero genetic distance between them, all

spatial analyses were performed on both the complete curtailed dataset, as well as a dataset from which all clones had been removed.

RESULTS

Genetic Diversity

Overall, genetic diversity of *Symbiodinium* within and among localities was high. Allelic richness varied broadly across loci, ranging from 7 to 89 (Table 3.2). Allele frequencies within localities ranged from 0.01 (recovered only once) to 1.0 (fixed). Most localities had an average haploid diversity between 0.40-0.79 (Table 3.2), although Bimini (BAH7), a particularly deep-water site in the Bahamas, composed mostly of clonal haplotypes, had a diversity of only 0.04. Across the range, gene diversity was negatively correlated with latitude (Figure 3.2, R^2=0.18, p=0.0117).

Surveys of intracolony variation of *Symbiodinium* showed identical haplotypes at all five sampling points within each of the 30 *G.ventalina* colonies sampled. The large majority of *G. ventalina* colonies sampled (n=1341, 81.8%) hosted only a single haplotype of *Symbiodinium*. Among colonies with more than one allele per locus, over half had multiple alleles at only one (n=108, 36.1%) or two (n=48, 16.1%) loci. The occurrence of multiple alleles at a given locus within individual *G. ventalina* colonies was not correlated across loci. In fact, contravening trends across localities were commonly observed, with some loci exhibiting the highest frequency of cooccurring alleles at sites where other loci had none, and visa versa. However, there was a weak but highly significant positive correlation between the allelic diversity of a locus at a given locality and the number of individuals with multiple alleles at that locus (R^2=0.05, p<0.0001). Exact tests of genic differentiation showed that alleles cooccuring within individual *G. ventalina* colonies were distributed identically to

Table 3.2: Haploid diversity (Nei's expected heterozygosity) and allelic richness, rarefaction corrected to n=10 (see text for details) for each locus (columns) at each locality (rows), and averaged over all loci and all localities. Site codes are given in Table 3.1. Microsatellite loci are described in Chapter 2 and Andras *et al*. 2009.

	GV2_100	GV2	GV42	GV44	SYM155	SYM254	SYM7	GV1C	GV2_70	GV28	Locality Mean
FLA1	0.84 / 5.2	0.59 / 2.6	0.1 / 1.4	0 / 1	0.84 / 5.3	0.79 / 4.6	0.05 / 1.2	0.86 / 5.5	0.9 / 6.6	0.31 / 2.1	**0.52 / 3.5**
FLA2	0.84 / 5.0	0.43 / 2.1	0.04 / 1.2	0 / 1	0.89 / 6.1	0.76 / 4.9	0.04 / 1.1	0.86 / 5.5	0.95 / 7.8	0.52 / 3.0	**0.53 / 3.8**
FLA3	0.75 / 4.1	0.26 / 1.9	0 / 1.0	0 / 1	0.82 / 4.9	0.77 / 3.8	0.03 / 1.0	0.81 / 4.5	0.92 / 7.0	0.49 / 2.6	**0.48 / 3.2**
FLA4	0.83 / 5.1	0.5 / 2.8	0.15 / 1.5	0 / 1	0.88 / 6.1	0.88 / 5.5	0.27 / 1.7	0.89 / 6.0	0.95 / 8.2	0.69 / 3.4	**0.60 / 4.1**
FLA5	0.85 / 5.7	0.47 / 3.0	0.17 / 1.6	0 / 1	0.88 / 6.3	0.86 / 5.9	0.19 / 1.6	0.9 / 6.5	0.96 / 8.3	0.73 / 3.6	**0.60 / 4.3**
FLA6	0.86 / 5.6	0.42 / 2.7	0.37 / 1.9	0 / 1	0.87 / 5.7	0.83 / 5.1	0 / 1	0.85 / 5.7	0.94 / 7.6	0.64 / 3.1	**0.57 / 3.9**
FLA7	0.89 / 5.9	0.25 / 2.0	0.07 / 1.4	0 / 1	0.88 / 5.9	0.83 / 5.0	0.38 / 1.9	0.87 / 5.9	0.96 / 7.7	0.56 / 2.8	**0.56 / 3.9**
FLA8	0.86 / 5.5	0.42 / 2.2	0.14 / 1.5	0 / 1	0.92 / 6.5	0.81 / 5.0	0.26 / 1.8	0.81 / 5.4	0.96 / 7.7	0.4 / 1.9	**0.55 / 3.8**
MBR1	0.81 / 5.0	0.51 / 2.7	0.31 / 2.0	0.46 / 2.7	0.73 / 4.2	0.28 / 2.2	0.42 / 1.9	0.69 / 3.9	0.77 / 4.2	0.65 / 3.1	**0.56 / 3.2**
MBR2	0.89 / 6.0	0.84 / 5.3	0.73 / 4.2	0.87 / 5.4	0.84 / 6.5	0.9 / 6.4	0.26 / 2.8	0.88 / 5.7	0.8 / 5.2	0.54 / 3.5	**0.75 / 5.1**
MBR3	0.82 / 4.4	0.69 / 2.9	0 / 1	0.51 / 2.7	0.84 / 4.4	0.76 / 4.2	0 / 1	0.71 / 2.9	0.53 / 3.2	0.6 / 2.7	**0.54 / 2.9**
MBR4	0.68 / 3.5	0.78 / 4.5	0.59 / 2.7	0.68 / 3.7	0.79 / 5.0	0.72 / 4.1	0.5 / 2.1	0.66 / 3.6	0.26 / 2.1	0.57 / 2.5	**0.62 / 3.4**
MBR5	0.84 / 5.4	0.82 / 4.8	0.62 / 2.7	0.8 / 5.3	0.76 / 4.1	0.9 / 6.5	0.05 / 1.2	0.86 / 5.6	0.82 / 4.9	0.64 / 3.5	**0.71 / 4.4**
MBR6	0.89 / 5.9	0.81 / 4.7	0.4 / 2.6	0.87 / 5.7	0.87 / 5.5	0.83 / 5.5	0.07 / 1.3	0.78 / 4.1	0.73 / 4.1	0.5 / 2.4	**0.67 / 4.2**
MBR7	0.87 / 5.7	0.74 / 4.1	0.44 / 2.9	0.87 / 5.7	0.81 / 4.7	0.77 / 4.8	0.21 / 1.8	0.66 / 3.6	0.79 / 4.5	0.35 / 2.3	**0.65 / 4.0**
MBR8	0.79 / 4.9	0.57 / 2.9	0.57 / 2.6	0.59 / 3.2	0.82 / 5.1	0.5 / 2.9	0.55 / 2.3	0.77 / 4.7	0.69 / 3.9	0.64 / 3.4	**0.64 / 3.6**
MBR9	0.86 / 5.8	0.81 / 5.0	0.62 / 3.2	0.73 / 4.5	0.79 / 5.1	0.86 / 5.8	0.62 / 3.0	0.89 / 6.1	0.8 / 5.0	0.74 / 4.0	**0.77 / 4.8**
MBR10	0.78 / 4.4	0.26 / 2.2	0.34 / 2.4	0.42 / 2.3	0.55 / 2.8	0.52 / 2.4	0.18 / 1.7	0.71 / 3.6	0.27 / 2.2	0.27 / 2.2	**0.43 / 2.6**
MBR11	0.58 / 3.7	0.5 / 2.9	0.62 / 3.5	0.44 / 3.0	0.44 / 2.8	0.57 / 3.5	0.54 / 2.8	0.57 / 3.6	0.51 / 3.1	0.54 / 2.8	**0.53 / 3.2**
MBR12	0.91 / 6.7	0.86 / 5.7	0.76 / 4.1	0.88 / 5.8	0.84 / 5.3	0.76 / 4.6	0.49 / 2.2	0.88 / 5.8	0.8 / 4.9	0.49 / 2.7	**0.76 / 4.8**
PAN1	0.88 / 6.0	0.88 / 5.7	0.48 / 3.0	0.8 / 4.6	0.88 / 5.6	0.86 / 5.5	0.38 / 2.6	0.86 / 5.7	0.79 / 5.4	0.79 / 5.1	**0.76 / 4.9**
PAN2	0.8 / 4.8	0.43 / 3.0	0.62 / 2.7	0.57 / 3.3	0.77 / 4.5	0.88 / 5.5	0.41 / 2.5	0.42 / 2.7	0.43 / 3.0	0.73 / 3.7	**0.60 / 3.6**
CUR	0.78 / 5.0	0.75 / 3.9	0.67 / 3.1	0.73 / 4.1	0.89 / 6.4	0.88 / 6.4	0.66 / 2.9	0.87 / 5.9	0.9 / 6.8	0.81 / 4.8	**0.79 / 4.9**
TOB	0.62 / 3.3	0.59 / 2.8	0.61 / 2.7	0.63 / 2.8	0.85 / 5.3	0.83 / 5.1	0.28 / 2.0	0.79 / 4.9	0.86 / 5.8	0.32 / 2.3	**0.63 / 3.7**
BAR	0.47 / 3.0	0.72 / 4.0	0.55 / 2.4	0.54 / 3.1	0.93 / 7.2	0.95 / 8.0	0.53 / 2.4	0.9 / 6.5	0.96 / 8.2	0.71 / 3.4	**0.72 / 4.8**
PRO	0.8 / 4.6	0.67 / 4.0	0.08 / 1.3	0.61 / 3.1	0.65 / 3.3	0.82 / 4.9	0 / 1	0.75 / 4.7	0.82 / 4.8	0.37 / 2.3	**0.55 / 3.4**
BAH1	0.78 / 4.5	0.8 / 4.6	0.07 / 1.3	0.59 / 3.6	0.61 / 3.4	0.77 / 4.7	0.43 / 2.5	0.88 / 5.8	0.86 / 5.4	0.56 / 2.7	**0.63 / 3.9**
BAH2	0.86 / 5.5	0.62 / 2.8	0.26 / 1.8	0.53 / 2.4	0.53 / 3.0	0.65 / 3.6	0.26 / 1.8	0.68 / 3.3	0.91 / 6.2	0.63 / 3.0	**0.59 / 3.3**
BAH3	0.69 / 3.4	0.49 / 2.5	0 / 1	0.64 / 3.3	0.54 / 2.5	0.49 / 2.5	0 / 1	0.74 / 3.6	0.59 / 3.2	0.4 / 1.9	**0.45 / 2.5**
BAH4	0.89 / 5.1	0.73 / 3.6	0.2 / 1.7	0.76 / 4.2	0.64 / 2.7	0.67 / 4.0	0.2 / 1.7	0.73 / 3.6	0.96 / 6.4	0.76 / 4.2	**0.65 / 3.7**
BAH5	0.8 / 4.8	0.69 / 3.9	0 / 1	0.65 / 3.1	0.74 / 4.3	0.73 / 3.9	0 / 1	0.65 / 3.6	0.9 / 6.5	0.56 / 2.8	**0.57 / 3.5**
BAH6	0.64 / 3.4	0.58 / 2.9	0.11 / 1.4	0.67 / 3.2	0.55 / 3.3	0.67 / 3.2	0.11 / 1.4	0.59 / 3.2	0.9 / 5.9	0.7 / 3.3	**0.55 / 3.1**
BAH7	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0 / 1	0.44 / 1.9	**0.04 / 1.0**
BER1	0.79 / 5.2	0.43 / 2.1	0.04 / 1.2	0.04 / 1.2	0.68 / 4.4	0.75 / 4.6	0.04 / 1.2	0.82 / 5.8	0.92 / 7.1	0.37 / 2.2	**0.48 / 3.5**
BER2	0.94 / 7.6	0.77 / 4.1	0.38 / 2.5	0 / 1	0.9 / 6.7	0.89 / 6.2	0.52 / 2.6	0.94 / 7.5	0.97 / 8.4	0.69 / 3.5	**0.7 / 5.0**
Locus Mean	**0.77 / 4.9**	**0.59 / 3.4**	**0.31 / 2.1**	**0.45 / 2.9**	**0.74 / 4.7**	**0.73 / 4.6**	**0.25 / 1.8**	**0.75 / 4.7**	**0.77 / 5.5**	**0.56 / 3.0**	

Grand mean (over all loci and localites) = 0.60 / 3.8

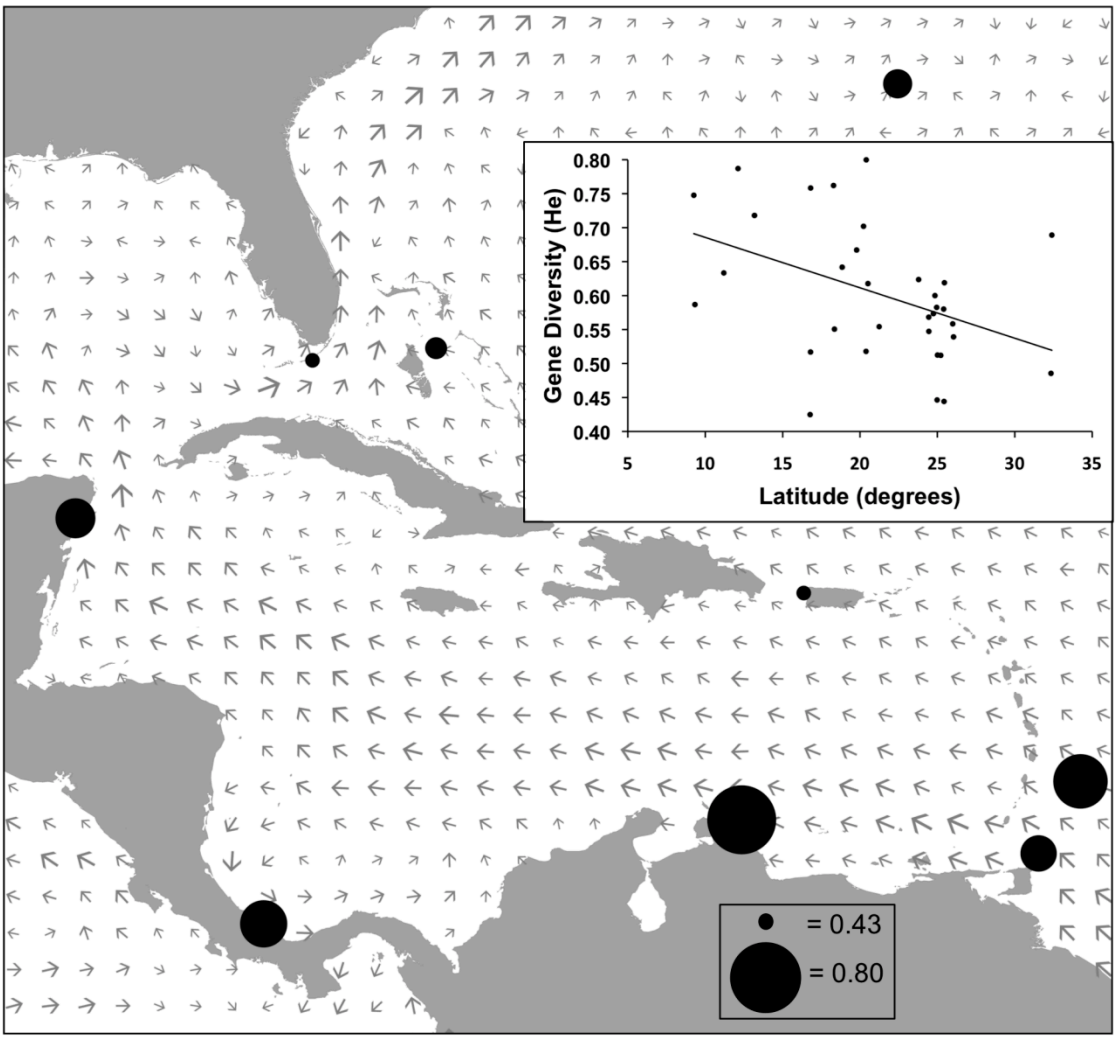

Figure 3.2: Geographic distribution of genetic diversity among *Symbiodinium* hosted by *G. ventalina*. Diameter of filled circles on map is scaled to the gene diversity (expected heterozygosity) of each sampling region. Inset plot depicts linear regression of gene diversity versus latitude (R^2=0.183, p=0.0117). Arrows on map illustrate average annual ocean currents, oriented to direction scaled to relative velocity, at 1x1 degree intervals (Ocean current data used with permission from Mariano et al. 1995).

alleles occurring individually, and allelic richness was identical regardless of whether samples with cooccurring alleles were included (full dataset) or not (curtailed dataset).

A total of 1162 multilocus haplotypes were scored from the 1341 individual *G. ventalina* colonies in the curtailed dataset. A large majority of these haplotypes (n=1056, 90.8%) were singletons unique to only one colony. Despite this substantial diversity, however, highly significant clonal population structure was observed across the entire dataset and within each of the sample localities (Table 3.1). A total of 106 haplotypes were shared by more than one colony. Of these, 78 (73.6%) were shared by only one other colony, and the rest were shared between 2 and 10 other colonies. Geographically, the large majority of clonal diversity (96.9%) was partitioned within localities. Most duplicate haplotypes were shared among colonies within the same locality (n=93, 87.7%), with the rest shared between adjacent localities ranging from 3-336 km away (mean = 37 km).

Population Structure

Estimates of F_{st} for the curtailed dataset ranged from 0.02 to 0.72 (mean=0.315), and more than 99% of all pairwise comparisons between localities were highly significant (Table 3.3, below diagonal). This pattern was nearly identical to those from the full dataset (Appendix Table 3.1). R_{st} values ranged from 0.01 to 0.98 (mean=0.732), and a smaller, though still large, majority of pairwise comparisons between localities were significantly different (94.6%, Table 3.3, above diagonal). All AMOVAs detected significant partitioning of genetic variance among regions, among localities within regions, and among individuals within localities (Table 3.4). Both the curtailed dataset and the complete dataset produced nearly identical results when analyzed with an infinite allele model, showing the majority of variation occurring within localities (68.48% - curtailed; 69.89% - full) and much lower proportions of

variation among reefs (13.98% - curtailed; 11.61% - full) and regions (17.54% - curtailed; 18.50% - full). However, applying a stepwise mutation model to the curtailed dataset yielded a nearly opposite distribution, with a majority of variance partitioned among regions (67.10%), and less within and among localities (26.77% and 6.13%, respectively).

The assumption of linkage equilibrium (LD) necessary for Bayesian clustering analysis was largely met. In the curtailed dataset, 22% of the 1583 pairwise comparisons per locus per population deviated significantly from linkage equilibrium. However, this fraction was reduced to just 9% after duplicate genotypes were removed from the dataset. The remaining LD was not limited to any particular pairs of loci and was largely restricted to localities with significant clonal structure. It was thus assumed that the remaining LD was due to nearly clonal variants resulting from limited recombination or mutation of existing clonal lineages.

Bayesian clustering analysis identified several well-defined, geographically-distinct clusters distributed across multiple hierarchical scales. At the uppermost level of population structure, two clusters were perfectly segregated based on geography - one group containing all individuals from the Florida Keys and Bermuda localities, the other group containing all individuals from the rest of the Caribbean. The second round of the ΔK method divided the Florida/Bermuda group into two clusters and split the rest of the Caribbean into four additional regional clusters, for a total of K=6. Four of the six clusters were not subdivided further by additional rounds of ΔK. Two clusters, primarily distributed across the Mesoamerican Barrier Reef and Panama, were each divided in two by a third round of the ΔK method, but the resulting subclusters tended to divide individuals within sampling localities as frequently as among sampling localities and generally failed to correspond in any obvious way with

geography or depth. Consequently, we chose a range-wide total of K=6 as the best and most sensible number of populations.

In the K=6 solution, a total of 1193 individuals (89%) were assigned to one of the six clusters, with a probability of at least 0.6 (Figure 3.3A and 3.3B). The six clusters were generally distributed cohesively across contiguous or adjacent localities and appeared to loosely delimit four broad intrinsically connected regions (Figure 3.3C). The Florida Keys and Bermuda shared two clusters, the first consisting mostly of individuals from the Upper and Lower Florida Keys (Figure 3.3, Purple Cluster), the second consisting mostly of individuals from the Middle Florida Keys and Bermuda localities (Figure 3.3, Blue Cluster). A third cluster united the large majority of individuals from Puerto Rico and the Bahamas (Figure 3.3, Green Cluster), and the fourth was limited to the Winward Islands of Curacao, Tobago, and Barbados (Figure 3.3, Orange Cluster). The remaining two clusters were distributed predominantly among localities along the Mesoamerican Barrier Reef and Panama in the Western Caribbean (Figure 3.3, Red & Yellow Clusters).

Within a reef, symbiont samples collected from the two $1m^2$ plots separated by ~14m showed no discernable spatial structure. However, STRUCTURE identified two genetic clusters segregated almost entirely according to size/age class of the *G. ventalina* host colony (Figure 3.4).

Many of the patterns observed in the Bayesian clustering analyses were also evident in the UPGMA dendrogram of genetic distances (Figure 3.5). Most localities from the same region tended to form exclusive or nearly exclusive groups that correspond well with the broad regional populations identified by STRUCTURE. Namely, the Florida Keys and Bermuda formed an exclusive group, as did the Bahamas/Puerto Rico, and the Winward Islands. Most of the localities from the Mesoamerican Barrier Reef were distributed between two exclusive groups.

Spatial Analyses

Genetic differentiation (F_{st}) among localities was positively correlated with geographic distance (Mantel test : r=0.38, p=0.001), illustrating a strong pattern of isolation by distance extending over the entire sample range. Figure 3.6 shows a plot of pairwise estimates of F_{st} versus the natural log of geographic distance among sampling localities. An overall positive correlation is evident, yet there are three noteworthy regions whose F_{st} values appear to weaken the trend of isolation by distance. First, F_{st} estimates between Bimini, in the Bahamas, and all other locations are quite high (mean =0.58), particularly in comparisons with other Bahamas localities which are, on average, less than 200 km away. Bimini is, by far, the deepest site sampled (21-24 m), indicating that substantial isolation occurs over depth, and this isolation is proportionally greater than isolation by distance. Second, pairwise comparisons between localities in the Bahamas and Florida Keys are also relatively high (mean=0.41), reinforcing the Bayesian clustering results which identified high differentiation between these regions despite their proximity. Conversely, comparisons between Bermuda and the Florida Keys are relatively low (mean=0.1) given the large distance between them. This observation also supports the Bayesian clustering results, which assigned many individuals from Florida and Bermuda to a common cluster.

Analyses of spatial autocorrelation also identified significant positive genetic structure, and provide a more detailed view of how this structure changes over increasing geographic distance. A correlogram of the autocorrelation coefficient (*r*) across a range of fixed distance classes (Figure 3.7A) shows the highest degree of spatial autocorrelation at the shortest distance class (50 km), decreasing thereafter and passing through zero at 250 km. This distance, at which *r* first crosses the x-axis, provides an estimate of the extent of positive genetic structure (Sokal and Wartenberg

1983, Smouse and Peakall 1999, Peakall et al. 2003). The dispersal neighborhood, defined as the region throughout which the larval pool is well-mixed, can be estimated from the autocorrelogram as the region over which the autocorrelation coefficient (*r*) remains roughly constant, before it begins to decline (Epperson 2005). The autocorrelogram of *Symbiodinium* (Figure 3.7A) shows no such initial plateau, but begins a steep decline immediately after the first distance class. This pattern held for distance class sizes as low as 1 km (data not shown), the maximum resolution possible with this dataset, suggesting that most *Symbiodinium* propagules disperse less than 1 km from their parent colonies. At larger distance classes, the correlogram fluctuates between negative and positive values. Though the specific trajectory of the correlogram is typically not interpreted beyond the first x-intercept, this fluctuation is also consistent with positive spatial structure at shorter distance classes (Smouse and Peakall 1999).

A plot of the autocorrelation coefficient across a range of increasing distance class sizes is shown in Figure 3.7B. This approach, which is less sensitive to distance class size, provides an alternative estimate of the extent of positive spatial genetic structure (Peakall et al. 2003). Autocorrelation is, again, highest for the smallest distance class and decreases steadily to near-zero at a distance class size of 900 km. Thereafter, *r* decreases much more slowly and does not become insignificant until a distance class size of 2500 km. Based on these two alternative analyses of spatial autocorrelation, the limit of positive spatial genetic structure for *Symbiodinium* hosted by *G. ventalina* is estimated to occur between 250 and 900 km.

Table 3.3: Average pairwise Fst values (below diagonal) and Rst values (above diagonal) among localities based on the curtailed multilocus haplotype data set (see text for detail). Site codes are given in Table 3.1. Non-significant values are shaded gray.

SITE	FLA1	FLA2	FLA3	FLA4	FLA5	FLA6	FLA7	FLA8	MBR1	MBR2	MBR3	MBR4	MBR5	MBR6	MBR7	MBR8	MBR9	MBR10	MBR11	MBR12	PAN1	PAN2	CUR	TOB	BAR	PRO	BAH1	BAH2	BAH3	BAH4	BAH5	BAH6	BAH7	BER1	BER2
FLA1	-	.02	.06	.05	.33	.60	.24	.39	.90	.90	.93	.88	.92	.82	.87	.89	.85	.94	.88	.85	.63	.81	.39	.71	.48	.86	.87	.79	.85	.81	.86	.81	.82	.82	.23
FLA2	.05	-	.05	0	.21	.50	.14	.26	.88	.88	.90	.86	.9*	.78	.84	.87	.82	.92	.85	.82	.58	.77	.36	.67	.44	.83	.83	.74	.80	.75	.81	.76	.76	.77	.16
FLA3	.07	.02	-	.14	.46	.70	.40	.55	.92	.92	.96	.89	.94	.82	.88	.91	.86	.96	.90	.86	.61	.82	.39	.71	.48	.92	.92	.83	.91	.87	.91	.86	.90	.85	.28
FLA4	.07	.03	.04	-	.16	.47	.14	.25	.89	.89	.91	.87	.91	.77	.84	.87	.82	.93	.85	.82	.55	.75	.34	.64	.40	.85	.85	.75	.82	.77	.84	.78	.79	.75	.13
FLA5	.08	.04	.06	0	-	.17	.16	.11	.91	.91	.93	.90	.92	.83	.88	.90	.86	.94	.89	.86	.67	.82	.41	.66	.40	.86	.87	.80	.85	.81	.86	.82	.82	.69	.03
FLA6	.15	.08	.12	.04	.02	-	.40	.24	.93	.93	.94	.92	.94	.87	.91	.93	.90	.95	.92	.90	.75	.87	.49	.68	.39	.90	.91	.86	.89	.86	.90	.87	.87	.60	.04
FLA7	.08	.06	.08	.04	.05	.10	-	.04	.89	.89	.91	.87	.91	.76	.84	.88	.82	.93	.85	.82	.53	.73	.26	.56	.30	.89	.85	.73	.81	.75	.84	.77	.79	.72	.07
FLA8	.08	.06	.08	.05	.06	.11	.02	-	.91	.91	.92	.89	.93	.78	.86	.89	.84	.94	.87	.84	.58	.76	.28	.54	.27	.90	.87	.76	.84	.78	.86	.80	.82	.66	.02
MBR1	.38	.38	.41	.32	.32	.33	.34	.35	-	0	.73	.31	.66	.44	.52	.01	.36	.19	.02	.21	.26	.24	.42	.77	.66	.95	.83	.73	.82	.79	.84	.79	.87	.93	.79
MBR2	.38	.38	.41	.32	.32	.33	.34	.35	0	-	.73	.31	.66	.44	.52	.01	.36	.19	.02	.21	.26	.24	.42	.77	.66	.95	.83	.73	.82	.79	.84	.79	.87	.93	.79
MBR3	.39	.38	.43	.33	.33	.34	.37	.38	.39	.39	-	.31	.24	.01	.03	.66	.20	.90	.55	.28	.16	.18	.29	.69	.56	.97	.84	.54	.81	.69	.87	.69	.96	.92	.76
MBR4	.36	.36	.39	.30	.30	.30	.34	.34	.26	.26	.16	-	.23	.14	.14	.25	.09	.53	.21	.03	.18	.10	.41	.78	.67	.90	.67	.58	.65	.64	.69	.64	.75	.93	.80
MBR5	.31	.30	.33	.26	.26	.26	.28	.29	.28	.28	.12	.17	-	.07	.06	.58	.07	.83	.56	.19	.17	.21	.40	.77	.65	.95	.77	.60	.75	.71	.79	.70	.86	.94	.80
MBR6	.32	.31	.35	.27	.27	.27	.30	.31	.32	.32	.04	.15	.05	-	0	.40	.07	.60	.31	.15	.10	.08	.26	.66	.56	.81	.33	.26	.31	.28	.36	.31	.45	.89	.71
MBR7	.33	.32	.35	.27	.26	.27	.31	.31	.33	.33	.06	.16	.09	.04	-	.45	.06	.68	.38	.14	.13	.11	.32	.72	.60	.88	.50	.39	.47	.46	.53	.46	.63	.91	.75
MBR8	.34	.33	.37	.28	.28	.28	.31	.31	.21	.21	.32	.26	.20	.26	.28	-	.28	.24	.02	.15	.21	.19	.42	.78	.66	.93	.78	.68	.76	.74	.80	.75	.83	.93	.79
MBR9	.29	.29	.32	.25	.25	.28	.25	.26	.22	.22	.21	.21	.12	.15	.16	.14	-	.57	.26	.04	.07	.04	.35	.73	.62	.86	.49	.40	.47	.45	.52	.47	.61	.91	.75
MBR10	.47	.47	.49	.41	.41	.41	.45	.45	.32	.32	.52	.38	.40	.44	.44	.15	.31	-	.13	.43	.40	.42	.47	.80	.69	.98	.93	.84	.92	.90	.94	.89	.96	.95	.82
MBR11	.41	.40	.44	.34	.34	.34	.38	.38	.28	.28	.41	.31	.31	.33	.34	.13	.23	.06	-	.15	.18	.11	.33	.71	.60	.93	.74	.60	.70	.64	.75	.68	.78	.91	.74
MBR12	.26	.25	.28	.19	.20	.20	.22	.22	.20	.20	.22	.18	.14	.16	.18	.12	.12	.20	.14	-	.11	.05	.38	.75	.64	.86	.58	.49	.55	.53	.59	.54	.66	.91	.76
PAN1	.27	.26	.30	.22	.22	.21	.24	.25	.22	.22	.20	.20	.12	.13	.17	.18	.13	.35	.26	.12	-	.04	.21	.61	.50	.64	.17	.10	.12	.09	.16	.13	.20	.81	.56
PAN2	.37	.36	.40	.32	.32	.33	.35	.34	.27	.27	.35	.29	.26	.29	.29	.24	.24	.39	.31	.20	.20	-	.23	.64	.53	.82	.36	.29	.32	.28	.39	.34	.46	.88	.68
CUR	.28	.27	.30	.21	.21	.22	.24	.24	.25	.25	.23	.22	.19	.20	.20	.19	.16	.31	.23	.14	.14	.24	-	.23	.22	.42	.14	.14	.11	.07	.13	.13	.09	.61	.35
TOB	.34	.34	.38	.32	.32	.35	.32	.32	.34	.34	.36	.33	.28	.29	.31	.31	.23	.43	.36	.27	.24	.34	.18	-	.09	.74	.62	.60	.59	.54	.61	.59	.54	.68	.53
BAR	.30	.29	.33	.25	.26	.27	.27	.27	.29	.29	.30	.29	.26	.26	.27	.24	.21	.34	.27	.20	.21	.29	.11	.17	-	.53	.46	.44	.41	.37	.43	.42	.35	.39	.30
PRO	.44	.44	.47	.40	.40	.41	.42	.43	.35	.35	.41	.37	.34	.36	.37	.35	.29	.46	.41	.29	.27	.37	.25	.35	.30	-	.93	.80	.92	.87	.92	.84	.95	.89	.65
BAH1	.39	.38	.41	.33	.34	.34	.35	.34	.32	.32	.36	.31	.26	.29	.30	.24	.20	.39	.33	.20	.18	.31	.20	.30	.26	.24	-	.11	.05	.16	.14	.17	.64	.90	.66
BAH2	.41	.41	.45	.37	.37	.38	.39	.39	.37	.37	.38	.34	.28	.30	.32	.30	.25	.44	.37	.23	.23	.36	.22	.33	.28	.26	.11	-	.04	0	.03	0	.19	.87	.60
BAH3	.47	.47	.51	.43	.43	.43	.45	.45	.41	.41	.46	.38	.34	.37	.39	.34	.31	.48	.43	.27	.27	.42	.26	.38	.32	.32	.15	.05	-	.05	.03	.06	.45	.88	.61
BAH4	.40	.40	.45	.35	.36	.37	.37	.37	.34	.34	.34	.32	.25	.27	.31	.28	.22	.44	.36	.21	.17	.33	.19	.30	.25	.21	.13	.02	.08	-	.02	0	.24	.86	.56
BAH5	.44	.44	.47	.39	.39	.40	.40	.40	.36	.36	.40	.34	.29	.32	.35	.31	.27	.45	.39	.26	.22	.37	.23	.32	.29	.25	.16	.13	.16	.05	-	0	.55	.89	.63
BAH6	.44	.45	.47	.39	.40	.41	.41	.41	.38	.38	.41	.35	.31	.33	.36	.33	.28	.46	.40	.26	.23	.38	.24	.33	.29	.27	.17	.15	.17	.08	0	-	.19	.87	.60
BAH7	.59	.61	.63	.58	.56	.56	.63	.63	.56	.56	.72	.51	.50	.52	.55	.51	.45	.67	.65	.46	.43	.63	.42	.52	.46	.48	.51	.53	.61	.56	.54	.58	-	.86	.54
BER1	.18	.17	.18	.12	.13	.14	.14	.15	.40	.40	.43	.37	.33	.35	.36	.34	.33	.46	.41	.23	.29	.39	.27	.39	.32	.47	.39	.43	.48	.43	.44	.45	.65	-	.44
BER2	.09	.07	.10	.04	.05	.07	.05	.06	.25	.25	.29	.26	.23	.24	.25	.22	.19	.34	.27	.14	.18	.26	.17	.27	.20	.35	.27	.30	.35	.28	.32	.33	.51	.11	-

Table 3.4: Results from the AMOVA using both the IAM (F_{ST} values, above) and the SMM (R_{ST} values, below) for the curtailed data set (a) and the IAM for the full dataset (b). See text for detail.

	Data Set / Model Used	Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percent Variation	Fixation Indices
a)	Curtailed /	Among regions	7	985.73	Va = 0.770	17.54	F_{CT} = 0.175*
	IAM	Among localities within regions	27	684.56	Vb = 0.614	13.98	F_{SC} = 0.169*
		Among individuals within a locality	1289	3877.26	Vc = 3.001	68.48	F_{ST} = 0.315*
b)	Curtailed /	Among regions	7	848890.652	Va = 811.473	67.10	R_{CT} = 0.671*
	SMM	Among localities within regions	27	81549.770	Vb = 74.079	6.13	R_{SC} = 0.186*
		Among individuals within a locality	1289	417372.463	Vc = 323.796	26.77	R_{ST} = 0.732*
c)	Full /	Among regions	7	2372.229	Va = 1.666	18.50	F_{CT} = 0.185*
	IAM	Among localities within regions	27	1465.653	Vb = 1.045	11.61	F_{SC} = 0.142*
		Among individuals within a locality	1605	10098.386	Vc = 6.292	69.89	F_{ST} = 0.301*

* = $P < 0.001$

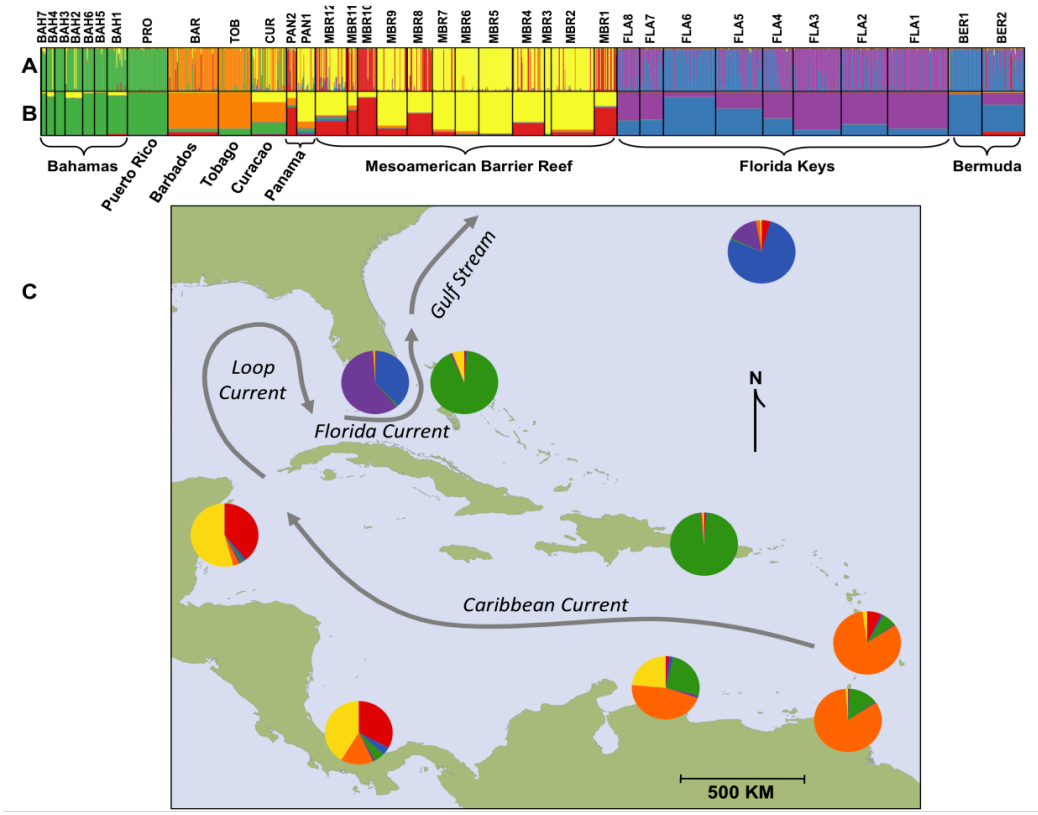

Figure 3.3: Bayesian clustering results for *Symbiodinium*, as calculated by the program STRUCTURE. Samples were assigned among six genetic clusters, which are coded by color (Red, Orange, Yellow, Green, Blue, Purple). Plot **A** portrays assignments of individual samples, where each thin horizontal line represents *Symbiodinium* from a single *Gorgonia ventalina* colony. Plot **B** illustrates average cluster assignments for each locality. Individuals are grouped by locality (codes given in Table 3.1) and listed in geographic order, beginning with the Bahamas and moving roughly clockwise around the range. Map **C** shows average assignments for each region as pie charts. Arrows denote major current patterns discussed in the text.

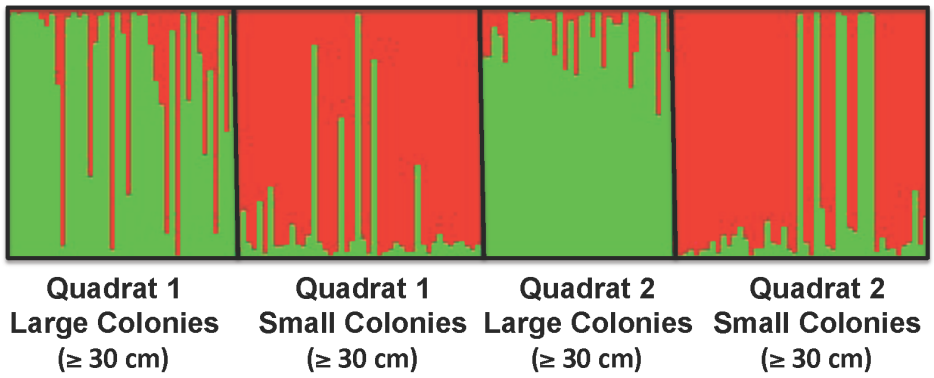

Figure 3.4: Bayesian clustering results for *Symbiodinium*, as calculated by the program STRUCTURE, for the meter-scale dataset. *Symbiodinium* populations were surveyed in 40 adult (>30cm) and 40 juvenile (<30cm) *Gorgonia ventalina* colonies from each of two quadrats, separated by ~14m. These size classes represent demographically non-overlapping groups. Individuals were assigned to two genetic clusters (red, green), which segregate largely based on size/age class but not by distance.

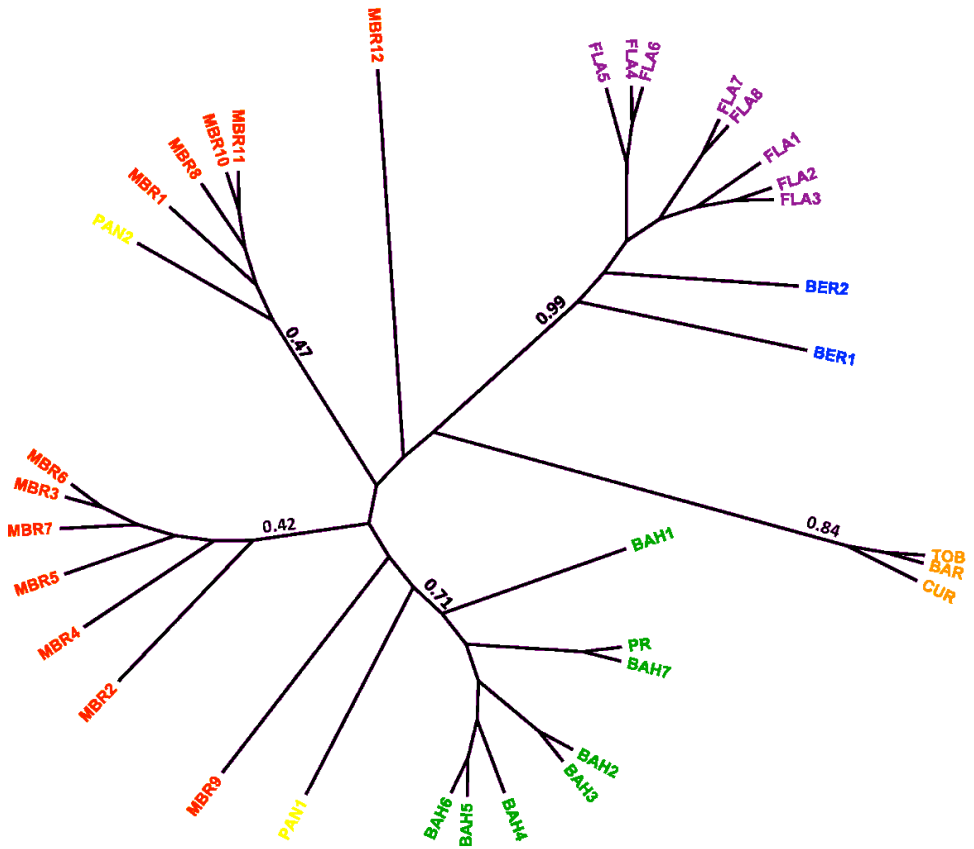

Figure 3.5: UPGMA dendrogram of Nei's unbiased genetic distance, depicting relationships among *Symbiodinium* populations of *Gorgonia ventalina* at 35 sites in the Caribbean and Western Atlantic. Localities are coded according to their dominant Bayesian cluster assignment (see Figure 2). Locality codes are given in Table 1. Bootstrap support of select nodes based on 100000 replicates.

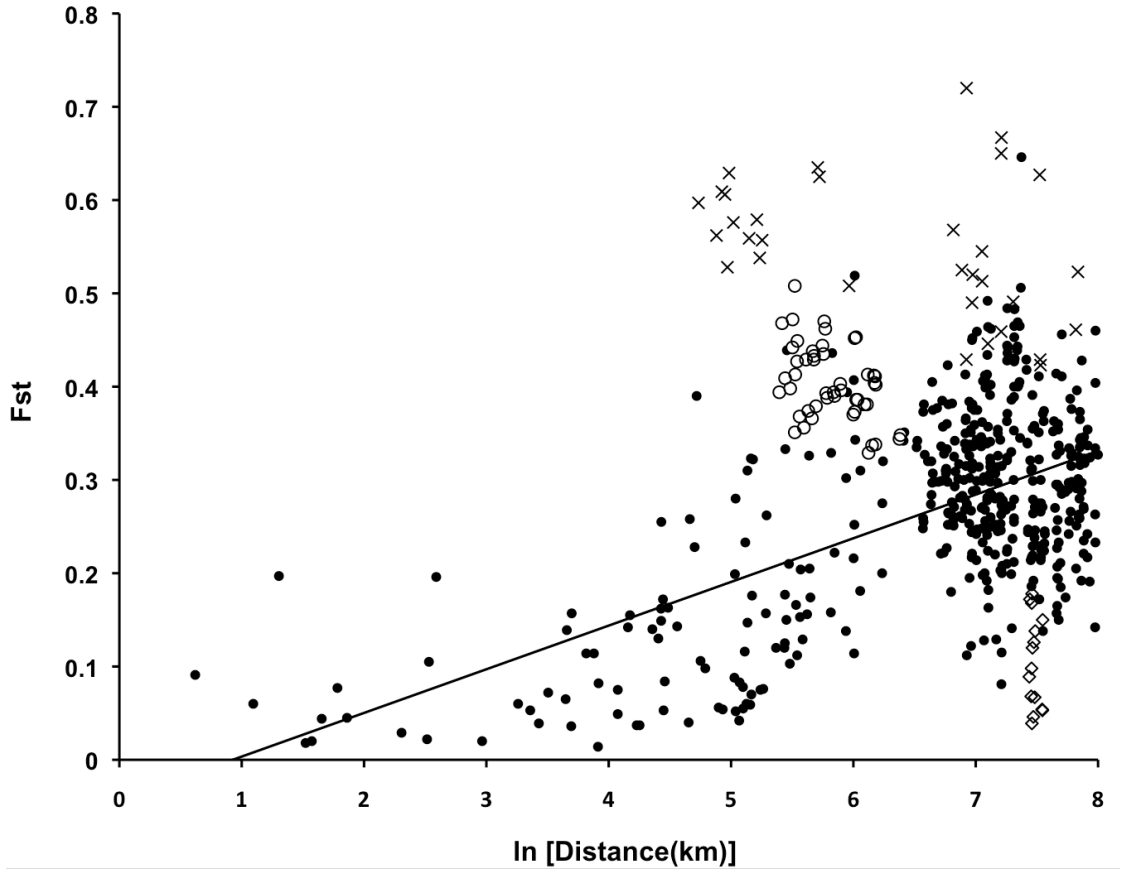

Figure 3.6: Plot of genetic differentiation (F_{st}) versus the natural log of the geographic distance among sampling localities. × = comparisons between Bimini (Bahamas) and all other localities. ○ = comparisons among Bahamas localities and Florida Keys localities. ◊ = comparisons among Bermuda localities and Florida Keys localities. ●= comparisons among all other localities. Distinguished groups are discussed in text. Linear trend-line is fit to entire dataset.

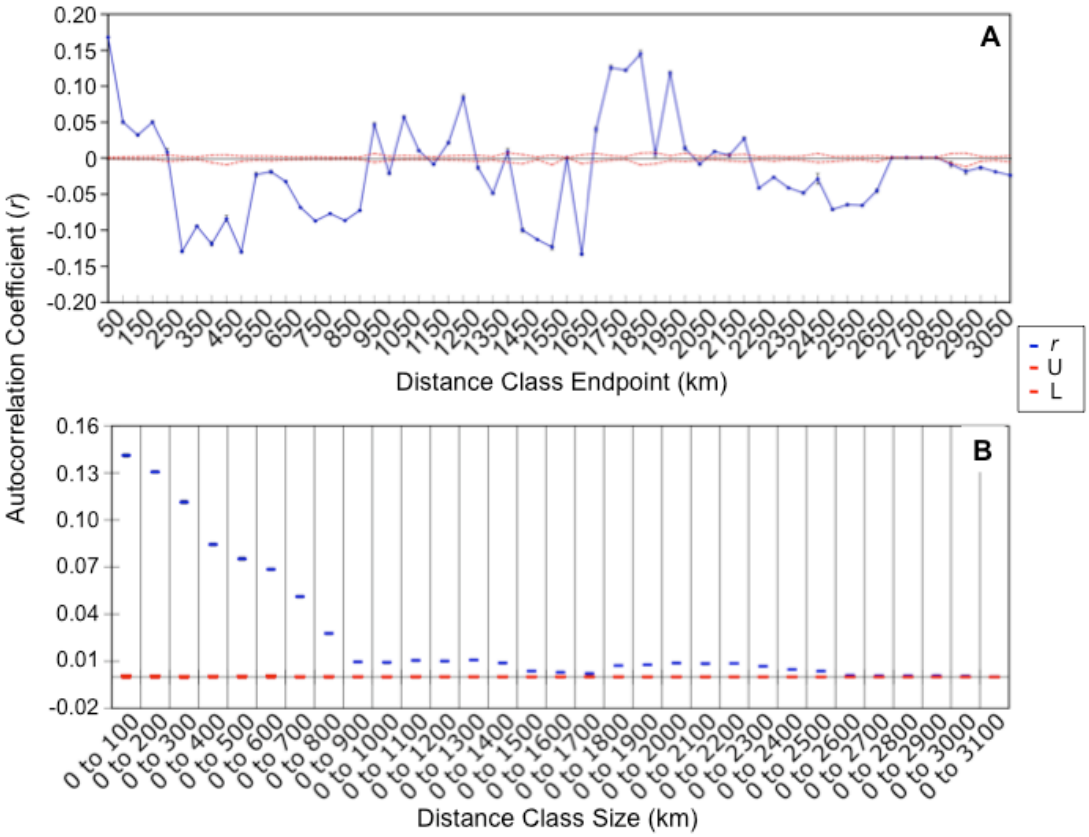

Figure 3.7: Spatial autocorrelation of genetic distance among *Symbiodinium*. **A**. Correlogram of the autocorrelation coefficient (*r*) over increasing distance classes of fixed size (50 km). **B.** Autocorrelation coefficient (*r*) over multiple distance classes of increasing size. Error bars about *r* (in blue) depict 95% confidence intervals based on 1000 bootstrap trials. U and L (in red - nearly overlapping so appear as only one mark) delimit the upper and lower bounds respectively of the 95% confidence interval about the null hypothesis of no spatial autocorrelation based on 1000 random permutations of the data.

DISCUSSION

This work substantially expands our understanding of the population biology of the most prevalent dinoflagellate symbiont in the Caribbean. Using 10 polymorphic microsatellites to investigate the population genetics of *Symbiodinium* B1/B184 throughout the entire range of its coral host, *Gorgonia ventalina*, we detected high levels of genetic diversity, significant differentiation over a broad range of spatial scales, and strong patterns of isolation by distance.

Genetic Diversity of Symbiodinium *B1/B184*

Both allelic and haplotypic diversity were remarkably high among individuals and populations of *Symbiodinium* B1/B184 (Table 3.2), as evidenced by the preponderance of unique haplotypes (90.8% of haplotypes). In contrast, most *G.ventalina* colonies hosted only a single *Symbiodinium* haplotype, indicating that intracolony diversity is generally low. This result may be an underestimate, as standard PCR-based genotyping techniques commonly fail to detect low-frequency strains of *Symbiodinium in hospite* (Santos *et al.* 2001). However, more than half of the ~20% of colonies that appeared to host more than one strain of *Symbiodinium* exhibited multiple genotypes at only one or two loci. To produce such patterns within a single host, multiple resident strains of *Symbiodinium* would either have to share alleles for at least eight of the ten loci (a very low probability occurrence, given the high allelic diversity), or null alleles would have to occur commonly enough to obscure true polymorphisms at a number of loci (also improbable, given the low frequency of null alleles). More plausibly, the occurrence of multiple alleles at just a few loci could be the result of errors or contamination in those PCR reactions or *in hospite* mutation. The presence of multiple *Symbiodinium* strains within a sample can

be more confidently identified when multiple alleles are observed at most or all loci. A more conservative estimate, based on a minimum criterion of observing multiple alleles for at least half of the loci, reduces the frequency of cooccurring strains of *Symbiodinium* to only ~4%. Previous studies using lower resolution markers such as ribosomal and chloroplast sequences have found that most coral colonies host a single dominant type of *Symbiodinium* (Baker 2003, LaJeunesse 2005). Yet, it is noteworthy that this pattern appears to extend to fine-scale diversity, given that *Symbiodinium* densities in healthy *G. ventalina* colonies can exceed $5x10^6$ per cm^2 (J. P. Andras, unpublished data), and a broad diversity of *Symbiodinium* haplotypes is presumably available in the environment. Such low intracolony diversity may be a consequence of the chance initiation of symbiosis with only one or a few *Symbiodinium* strains. Alternatively, it could be the result of fine-scale host/symbiont specificity. Coffroth et al. (2001) found that newly settled polyps of the gorgonian coral *Plexaura kuna* non-selectively acquired a broad diversity of *Symbiodinium* clades, which were largely reduced to a single clade after the first three months of development. It would be interesting to determine whether such ontogenetic specificity occurs at much finer genetic scales, which could explain the low intracolony diversity observed in this study.

Across the range of *G. ventalina*, gene diversity of *Symbiodinium* was variable and correlated negatively with latitude (Figure 3.2). This trend is driven, in part, by the low diversity of oceanographically "downstream" localities such as the Bahamas, Florida Keys, and Bermuda, at the absolute northern limit of *G. ventalina*'s distribution. The combination of isolation, extreme environmental conditions, and genetic drift associated with low population densities are expected to result in reduced genetic diversity at the edges of species ranges (Hoffmann and Blows 1994). Although *Symbiodinium* do occur in temperate waters (Baker 2003), their densities are

much higher on reefs (Littman *et al.* 2008), and their distribution is likely centered in the tropics. Especially in light of their strong population structure, the low genetic diversity of higher latitude peripheral populations is not surprising.

Symbiodinium haplotypes within individual *G. ventalina* colonies were completely homogenous, even in colonies that were host to multiple haplotypes. Such homogeneity appears to be the predominant pattern among corals (Goulet 2006, Stat et al. 2006) and has been observed in at least one other species of octocoral (Goulet and Coffroth 2003). Moreover, the few known cases of intracolony *Symbiodinium* zonation occur in scleractinian corals, where algal clades are differentially distributed among irradiance microenvironments across the rigid colony surface (Rowan et al. 1997, van Oppen et al. 2001). In contrast, the skeleton of gorgonian corals like *G. ventalina* is flexible. These corals tend to reside in high-energy habitats where they are bent back and forth constantly by the surge, and average irradiance may therefore be similar across a colony's surface. This effective lack of microhabitats may explain the homogeneity of *Symbiodinium* within *G. ventalina* colonies. Alternatively, the lack of zonation may indicate that there are no significant functional differences among the fine-scale diversity of *Symbiodinium* measured in this study.

Despite the high haplotypic diversity of *Symbiodinium* B1/B184, signs of clonal reproduction were also observed. On average, nearly one in ten haplotypes was hosted by more than one *G. ventalina* colony. Nearly all of these duplicate haplotypes occurred within localities, suggesting low clonal dispersal between reefs. Clonal reproduction not only generates duplicate haplotypes, but can also reduce the diversity of non-identical haplotypes (Gomez and Carvalho 2000, Stenberg et al. 2003) and increase linkage disequilibrium among loci (Tibayrenc et al. 1991, Awadalla 2003) relative to expectations under random mating. Both of these patterns were evident in our dataset. Moreover, the observed linkage disequilibrium occurred primarily at

localities with the highest proportion of repeated haplotypes and was reduced substantially when those duplicates were removed. This pattern has been defined as "epidemic" population structure by Smith *et al*. (1993), and is interpreted as evidence of the recent expansion of certain clonal lineages amidst a broader population that is effectively panmictic. A similar pattern was observed in *Symbiodinium* B1/B184 hosted by the gorgonian coral *Pseudopterogorgia elisabethae* (Santos *et al.* 2003b), although the frequency of repeated haplotypes (73.9%) was much higher than the present study (9.1%). This disparity is unlikely due to actual differences in clonal frequency of the same *Symbiodinium* lineage as it occurs in different host species, but rather, is probably a consequence of differential discriminative power of the genetic markers used in each study. In cases where the number and/or diversity of genetic markers is low, repeated haplotypes can occur commonly by chance and may therefore not represent true clonal genomes (Halkett *et al.* 2005). The relatively high number of polymorphic loci used in this study are unlikely to be shared by non-clonemates and are thus more accurate measures of true clonal prevalence.

The observed linkage equilibrium among the majority of non-clonal haplotypes suggests that recombination, presumably via sexual reproduction, is common among this lineage of *Symbiodinium*. These results are supported by previous studies that have found similar molecular evidence for recombination (Baillie et al. 2000a, Baillie et al. 2000b, LaJeunesse 2001, Goulet and Coffroth 2003, Santos et al. 2003b). In culture, *Symbiodinium* are known to alternate between a haploid vegetative cyst (Santos and Coffroth 2003) and a motile sexual zoospore, which has been proposed to be diploid (Freudenthal 1962, Trench 1997). However, given that the zoospore stage of *Symbiodinium* has never been observed *in hospite*, sex likely occurs in the free-living life stage, about which we know very little.

Differentiation and Biogeography of Symbiodinium *B1/B184 Populations*

Populations of *Symbiodinium* B1/B184 hosted by *G. ventalina* were highly differentiated across a broad range of spatial scales (Table 3.3 and Table 3.4). This result was consistent for both the full dataset (alleles coded as dominant markers) and the curtailed dataset (alleles coded as codominant multilocus haplotypes), although the distribution of molecular variance was quite different depending on which mutational model was employed. Hierarchical AMOVAs based on the infinite allele model (IAM; F_{st}-based), which treats all allelic diversity as equivalent, partitioned roughly 70% of genetic variation among individuals *within localities* (Table 3.4A, 3.4C). Conversely, the AMOVA based on a stepwise mutation model (SMM; R_{st}-based), which assumes that the relatedness between alleles is inversely proportional to their difference in size, identified more than two-thirds of the genetic variation *among regions* (Table 3.4B). The SMM is thought to more realistically represent the actual microsatellite mutational mechanism of replication slippage (Slatkin 1995), and simulation studies have found that the SMM provides a more robust measure of differentiation than the IAM in cases of low gene-flow (Balloux and Goudet 2002), such as this study. Nonetheless, the contrasting results achieved by both models provide important information about the nature of differentiation among *Symbiodinium* populations. Namely, remarkable genetic diversity does occur within localities, but this diversity is primarily drawn from a distribution of relatively closely-related, similarly-sized alleles. Alternatively, diversity partitioned among distinct regions of the Caribbean tends to be composed of more distantly-related alleles of markedly different size.

Pairwise comparisons of F_{st} and R_{st} identified highly significant differentiation between nearly all localities (Table 3.3), from the nearest neighboring reefs (~1 km) to those most distantly separated (3124 km). The full and curtailed dataset produced

nearly identical F_{st} values (Table 3.3 below diagonal, Appendix Table 3.1). These results, along with the nearly identical allelic richness and frequency between the two datasets, indicate that cooccurring strains of *Symbiodinium* within a single *G. ventalina* colony are drawn from the same local population as individually occurring strains, and exclusion of these samples does not alter patterns of diversity or differentiation.

The degree of differentiation between localities, as measured by both F_{st} and R_{st}, was positively correlated with the geographic distance between them (Figure 3.6 and Figure 3.7), indicating that distance is an important isolating mechanism for *Symbiodinium*. This correlation held both within and across all regions, although the correlation was strongest for distances less than 900 km. Additionally, the UPGMA dendrogram of pairwise genetic distance (Figure 3.5) illustrates that sites from the same region tended to form exclusive or nearly exclusive groups, supporting the observation that genetic similarity is strongly associated with geographical proximity.

The results of the AMOVAs and F-statistics, which are based on differences in allele frequencies, were corroborated and extended by Bayesian clustering analyses, which are based on patterns of linkage equilibrium among loci. Bayesian clustering can resolve hierarchical genetic structure without *a priori* assumptions about an individual's population membership, and thus provides a less biased estimate of biogeography than frequentist population genetic measures such as F-statistics. At the highest level of hierarchical structure, our dataset was divided into two clusters perfectly segregated by geography, one consisting of all individuals from the Florida Keys and Bermuda, the other uniting the rest of the Caribbean. The isolation of Florida and Bermuda is oceanographically sensible, as these localities are separated from the rest of the Caribbean by significant hydrodynamic and geographic barriers (Figure 3.3). The Caribbean Current is a strong northwesterly surface current (Gordon

1967), which, along with its seasonally variable eddies and meanders, could provide a mechanism for gene flow among localities in and around the Caribbean Basin. As the Caribbean Current moves west and north through the Yucatan Channel, it enters the Loop Current, which extends in a clockwise arc into the Gulf of Mexico (Hofmann and Worley 1986). The Loop Current exits the Gulf of Mexico through the Florida Straight between Cuba and the Florida Keys, becoming the Florida Current (Schmitz and Richardson 1968). Extended entrainment in the Loop Current over deep water in the central Gulf of Mexico, could serve as a barrier to pelagic propagules leaving the Caribbean Basin. Furthermore, much of the flow leaving the Gulf of Mexico via the Florida Current passes south of the Keys and may never come into contact with reefs along the continental shelf. The Florida Current becomes quite strong as it moves through the Florida Straight and joins the Gulf Stream (Leaman *et al.* 1989), providing a likely barrier between the Florida Keys and the Bahamas, despite their close proximity.

At higher resolution, the two clusters from the first hierarchical level were split into a total of six clusters (Figure 3.3), which we accepted as our most sensible and final estimate of the number of populations. Two clusters (Blue and Purple) were composed almost entirely of individuals from the Florida Keys and Bermuda, with the majority of individuals from the Keys being assigned to the Purple Cluster, and most of Bermuda assigned to the Blue. However, there were significant exceptions to this general pattern. In particular, a substantial proportion of individuals from Middle and Lower Keys localities were assigned to the primarily-Bermudan Blue Cluster. This pattern is consistent with propagule dispersal via the Florida Current/Gulf Stream (Figure 3.3). Sea levels in the Gulf of Mexico and Florida Bay are, on average, higher than those in the Atlantic, creating a mean downstream flow that not only drives the Florida Current but also results in significant drainage from Florida Bay through tidal

channels between the Florida Keys (Smith 1994). The largest tidal channels connecting Florida Bay to the Atlantic occur in the Middle and Lower Keys, while nearly none are found in the Upper Keys (see Figure 3.1A). Drainage through these channels in the Middle and Lower Keys could entrain propagules from local reefs and carry them into the Florida Current. In support of this hypothesis, the Florida Keys localities exhibiting the highest degree of shared heritage with Bermuda were Alligator (FLA5) and Tennessee (FLA6) Reefs, which lie directly in front of three of the highest-flow tidal channels in the Keys (Long Key Channel, Channel 5, Channel 2). Nonetheless, the observed connectivity between the Florida Keys and Bermuda is surprising. Although the Florida Current/Gulf Stream could transport propagules from the Keys far to the North and East, Bermuda lies in the doldrums of the central Sargasso Sea (Bischof *et al.* 2004), and there are no obvious strong hydrodynamics that would connect Bermuda with the Florida Current. It is noteworthy that the relatively low differentiation between these regions, identified by both Bayesian clustering (Figure 3.3) and F_{st} (Figure 3.6), is largely due to haplotypes at a single locus. All individuals from both Florida and Bermuda are fixed for the same single allele at locus GV44, while all other localities are polymorphic at this locus. As it seems unlikely that large numbers of *Symbiodinium* would routinely disperse between these two distant localities given the strong local structure observed elsewhere, it is more likely that the observed affinity between Florida and Bermuda is a product of founder effect evident as a fixed rare allele, and not due to substantial ongoing gene flow. The low genetic diversity of Bermuda localities (Figure 3.2) supports this hypothesis.

The remaining four clusters were primarily distributed across contiguous or adjacent reefs in and around the Caribbean Basin, and appeared to roughly delineate three broad regional populations (Figure 3.3). In the Western Caribbean, individuals

from the Mesoamerican Barrier Reef and Panama were predominantly assigned to one of two clusters (Red or Yellow). These clusters appeared to be loosely segregated based on cross-shelf position, with the Red Cluster commonly occurring at offshore sites (MBR 1,4,8,10,11,12) and inshore sites dominated by the Yellow Cluster. Interestingly, even at sites where the Red and Yellow clusters co-occurred, most individuals were resolutely assigned to one or the other, suggesting that limited recombination occurs between them.

The large majority of individuals from all seven Bahamas localities were assigned to a common cluster (Green) along with all individuals from Puerto Rico. These results suggest that gene flow is substantial enough among localities in the Northern Caribbean and Bahamas to homogenize individuals at this level of genetic resolution, a result consistent with the variable and sometimes strong currents in that region (Johns *et al.* 2008). To the east of Puerto Rico, the Winward Islands of Barbados and Tobago were dominated by a single cluster (Orange), and showed little mixing with localities to the west. Previous studies of fish (Colin 2003, Taylor and Hellberg 2003, 2006) and corals (Baums et al. 2005, Baums et al. 2006, Galindo et al. 2006) have identified an east/west break near Puerto Rico, suggesting that this may be a common biogeographic divide for many Caribbean organisms. However, the exact placement of this break has not been consistently resolved, with some studies placing Puerto Rico to the east of this divide and others to the west. Our results resolutely group Puerto Rico with localities to the west, indicating that, for *Symbiodinium*, this break falls somewhere east of Puerto Rico.

In the southern central Caribbean basin, most samples from the island of Curacao were assigned to the Orange cluster indicating affinity with sites to the East. However, substantial admixture with the Yellow and Green Clusters was also evident, suggesting connectivity with the Western and Northern Caribbean. From the results

presented here, it is impossible to determine the directionality of this connectivity, but, based solely on prevailing current patterns, reefs in the southeastern Caribbean might be expected to serve as source populations. Most water flows into the Caribbean through channels between the Winward Islands (Johns *et al.* 2002), and the strongest currents in the Caribbean flow west/northwest along the Northern Coast of Venezuela near Curacao (Frantanoni 2001). Such strong currents could entrain propagules and carry them to "downstream" sites to the West and North.

Symbiodinium have a flagellated free-living life stage that is capable of active motility (Freudenthal 1962). Moreover, these dinoflagellates have been isolated from the water column (Coffroth et al. 2006, Littman et al. 2008, Manning and Gates 2008, Porto et al. 2008), fish feces (Muller Parker 1984, Porto et al. 2008), and ship ballast water (Stat *et al.* 2008), indicating that the potential for active, passive, and/or facilitated transport does exists. Nonetheless, our results, along with several others' (Santos et al. 2003b, Howells et al. 2009, Kirk et al. 2009), indicate a high degree of local endemism and little gene flow among reefs. Littman et al. (2008) have found that while *Symbiodinium* do occur in the water column, interstitial densities are many times higher, indicating that the free-living stage occurs primarily in the benthos. Moreover, this same study found that both benthic and pelagic densities of *Symbiodinium* dropped dramatically beyond the reef margin. The aggregation of *Symbiodinium* within reef habitat could be a result of positive chemotaxis toward hosts (Fitt 1984, Yacobovitch et al. 2004, Pasternak et al. 2006), or the constant release of symbionts by hosts (Jones and Yellowlees 1997, Baghdasarian and Muscatine 2000). Regardless, the restriction of *Symbiodinium* largely to sediments within a reef provides a plausible explanation for the patterns of strong local differentiation seen in this study, while their rarer presence in the water column could account for the low levels of observed connectivity.

Within a reef, *Symbiodinium* were not structured spatially across tens of meters, however, there was a clear distinction between populations hosted by *G. ventalina* of different age classes (Figure 3.4). In cases of vertical symbiont transmission, the provisioning of coral eggs or larvae with symbionts from the maternal colony can drive patterns of *Symbiodinium* population structure among hosts (Stat *et al.* 2008). Neither spawning nor newly settled juveniles have been reported for *G. ventalina*, and the mechanisms of reproduction and symbiont acquisition are therefore not definitively known. However, morphological and histological evidence (Petes et al. 2003, Fitzsimmons-Sosa et al. 2004), along with the discordance between host and symbiont population structure (J. P. Andras, unpublished data), strongly imply that symbiont transmission occurs horizontally in this mutualism and not via the coral larva. Consequently, the observed cohort effect is likely a result of the environmental dynamics of free-living *Symbiodinium* populations, and not due to spatiotemporally variable transport via host propagules. This pattern is consistent with the hypothesis of "epidemic" population structure, evidenced by the observed clonality and attendant patterns of linkage disequilibrium. *G. ventalina* juveniles are apparently infected by the locally predominant *Symbiodinium* strain which changes over time. The variability of available symbiont types could be due to a number of physical and biological factors such as currents or the release of *Symbiodinium* cells by local hosts. Alternatively, they may be due to the differential success of *Symbiodinium* strains best adapted to fluctuating selection regimes. Future work on the functional significance of fine-scale *Symbiodinium* diversity will help resolve these mechanisms.

CONCLUSIONS

Taken together, the results presented here suggest a general model for the population structure and dynamics of *Symbiodinium* B1/B184: Across the range of *G.*

ventalina, the distinct distribution of several contiguous *Symbiodinium* metapopulations delimits four broad regions (Florida/Bermuda; Northern Caribbean; Western Caribbean; Eastern Caribbean). Patterns of connectivity and isolation within and among these regions appear to correspond with known oceanographic and bathymetric features, suggesting that the limited connectivity observed may occur via ocean currents. Within regional metapopulations, populations are structured hierarchically, based in part on isolation by distance. Dispersal among reefs is low, leading to local endemism and high differentiation. Populations within a reef are genetically diverse and recombine via sexual reproduction. However, patterns of epidemic population structure are evident, whereby alternating pulses of expansion by different clonal lineages leave a population genetic signal of linkage disequilibrium. Within individual *G. ventalina* colonies, diversity is generally low and homogeneously distributed.

There are a number of key unknowns that prevent complete elucidation of the mechanisms governing the observed patterns. In particular, future investigations of potential functional diversity and specificity among fine-scale lineages of *Symbiodinium* will contribute greatly to our understanding of this system. If the diversity described here is a fine-scale analog of diversity at the clade or phylotype level, then it may play an important role in the ecology of the coral holobiont and provide an added dimension of adaptive flexibility in the face of escalating environmental perturbation.

APPENDIX

Appendix Table 3.1: Pairwise Fst values for all loci based on the full data set (below diagonal) and the curtailed data set (above diagonal). Data are coded as dominant markers based on the presence/absence of alleles; see text for detail. Site codes are given in Table 3.1. Non-significant values are shaded gray.

SITE	FLA1	FLA2	FLA3	FLA4	FLA5	FLA6	FLA7	FLA8	MBR1	MBR2	MBR3	MBR4	MBR5	MBR6	MBR7	MBR8	MBR9	MBR10	MBR11	MBR12	PAN1	PAN2	CUR	TOB	BAR	PRO	BAH1	BAH2	BAH3	BAH4	BAH5	BAH6	BAH7	BER1	BER2
FLA1	-	.05	.07	.07	.08	.15	.08	.08	.38	.38	.39	.36	.31	.32	.33	.34	.29	.47	.41	.26	.27	.37	.28	.34	.30	.44	.39	.41	.47	.40	.44	.44	.59	.18	.09
FLA2	.07	-	.02	.03	.04	.08	.06	.06	.38	.38	.38	.36	.30	.31	.32	.33	.29	.47	.40	.25	.26	.36	.27	.34	.29	.44	.38	.41	.47	.40	.44	.45	.61	.17	.07
FLA3	.09	.04	-	.04	.06	.12	.08	.08	.41	.41	.43	.39	.33	.35	.35	.37	.32	.49	.44	.28	.30	.40	.30	.38	.33	.47	.41	.45	.51	.45	.47	.47	.63	.18	.10
FLA4	.09	.05	.08	-	0	.04	.04	.05	.32	.32	.33	.30	.26	.27	.27	.28	.25	.41	.34	.19	.22	.32	.21	.32	.25	.40	.33	.37	.43	.35	.39	.39	.58	.12	.04
FLA5	.08	.04	.08	0	-	.02	.05	.06	.32	.32	.33	.30	.26	.27	.26	.28	.25	.41	.34	.20	.22	.32	.21	.32	.26	.40	.34	.37	.43	.36	.39	.40	.56	.13	.05
FLA6	.14	.09	.15	.03	.03	-	.10	.11	.33	.33	.34	.30	.26	.27	.27	.28	.28	.41	.34	.20	.21	.33	.22	.35	.27	.41	.34	.38	.43	.37	.40	.41	.56	.14	.07
FLA7	.09	.08	.11	.06	.05	.10	-	.02	.34	.34	.37	.34	.28	.30	.31	.31	.25	.45	.38	.22	.24	.35	.24	.32	.27	.42	.35	.39	.45	.37	.40	.41	.63	.14	.05
FLA8	.08	.07	.12	.07	.05	.10	.01	-	.35	.35	.38	.34	.29	.31	.31	.31	.26	.45	.38	.22	.25	.34	.24	.32	.27	.43	.34	.39	.45	.37	.40	.41	.63	.15	.06
MBR1	.36	.36	.41	.31	.30	.30	.31	.31	-	0.0	.39	.26	.28	.32	.33	.21	.22	.32	.28	.20	.22	.27	.25	.34	.29	.35	.32	.37	.41	.34	.36	.38	.56	.40	.25
MBR2	.25	.24	.30	.21	.20	.20	.22	.21	.13	-	.39	.26	.28	.32	.33	.21	.22	.32	.28	.20	.22	.27	.25	.34	.29	.35	.32	.37	.41	.34	.36	.38	.56	.40	.25
MBR3	.35	.35	.42	.30	.28	.30	.31	.32	.32	.09	-	.16	.12	.04	.06	.32	.21	.52	.41	.22	.20	.35	.23	.36	.30	.41	.36	.38	.46	.34	.40	.41	.72	.43	.29
MBR4	.36	.36	.42	.31	.30	.29	.33	.33	.23	.13	.15	-	.17	.15	.16	.26	.21	.38	.31	.18	.20	.29	.22	.33	.29	.37	.31	.34	.38	.32	.34	.35	.51	.37	.26
MBR5	.29	.29	.35	.25	.24	.24	.26	.26	.24	.04	.09	.17	-	.05	.09	.20	.12	.40	.31	.14	.12	.26	.19	.28	.26	.34	.26	.28	.34	.25	.29	.31	.50	.33	.23
MBR6	.32	.32	.38	.27	.26	.26	.28	.29	.28	.06	.03	.16	.05	-	.04	.26	.15	.44	.33	.16	.13	.29	.20	.29	.26	.36	.29	.30	.37	.27	.32	.33	.52	.35	.24
MBR7	.31	.30	.37	.25	.24	.24	.27	.27	.27	.07	.05	.14	.08	.04	-	.28	.16	.44	.34	.18	.17	.29	.20	.31	.27	.37	.30	.32	.39	.31	.35	.36	.55	.36	.25
MBR8	.35	.35	.41	.30	.29	.28	.31	.31	.18	.14	.30	.26	.21	.26	.25	-	.14	.15	.13	.12	.18	.24	.19	.31	.24	.35	.24	.30	.34	.28	.31	.33	.51	.34	.22
MBR9	.29	.29	.35	.26	.25	.27	.25	.25	.19	.08	.17	.19	.10	.13	.13	.14	-	.31	.23	.12	.13	.24	.16	.23	.21	.29	.20	.25	.31	.22	.27	.28	.45	.33	.19
MBR10	.47	.46	.51	.42	.40	.40	.43	.45	.29	.26	.50	.36	.38	.42	.40	.14	.30	-	.06	.20	.35	.39	.31	.43	.34	.46	.39	.44	.48	.44	.45	.46	.67	.46	.34
MBR11	.40	.39	.46	.34	.33	.32	.35	.36	.24	.18	.36	.29	.28	.30	.28	.11	.22	.06	-	.14	.26	.31	.23	.36	.27	.41	.33	.37	.43	.36	.39	.40	.65	.41	.27
MBR12	.26	.25	.32	.20	.20	.19	.21	.21	.17	.08	.17	.18	.12	.13	.14	.12	.10	.20	.13	-	.12	.20	.14	.27	.20	.29	.20	.23	.27	.21	.26	.26	.46	.23	.14
PAN1	.27	.27	.34	.23	.22	.21	.24	.24	.19	.07	.17	.20	.10	.12	.14	.19	.12	.35	.25	.10	-	.20	.14	.24	.21	.27	.18	.23	.27	.17	.22	.23	.43	.29	.18
PAN2	.38	.38	.44	.34	.32	.33	.34	.35	.25	.16	.34	.29	.26	.28	.27	.25	.24	.40	.31	.20	.21	-	.24	.34	.29	.37	.31	.36	.42	.33	.37	.38	.63	.39	.26
CUR	.28	.28	.34	.23	.22	.22	.25	.25	.24	.12	.22	.23	.18	.19	.18	.22	.17	.33	.25	.15	.13	.27	-	.18	.11	.25	.20	.22	.26	.19	.23	.24	.42	.27	.17
TOB	.34	.35	.41	.34	.32	.34	.32	.32	.33	.22	.34	.34	.28	.30	.30	.32	.23	.45	.36	.26	.23	.38	.16	-	.17	.35	.30	.33	.38	.30	.32	.33	.52	.39	.27
BAR	.30	.30	.35	.27	.26	.27	.28	.27	.28	.17	.27	.29	.23	.25	.24	.25	.19	.35	.28	.19	.18	.31	.09	.13	-	.30	.26	.28	.32	.25	.29	.29	.46	.32	.20
PRO	.43	.44	.49	.40	.39	.38	.40	.41	.32	.23	.37	.34	.31	.32	.33	.34	.27	.45	.39	.26	.25	.38	.25	.35	.28	-	.24	.26	.32	.21	.25	.27	.48	.47	.35
BAH1	.39	.38	.45	.34	.33	.33	.34	.33	.29	.18	.32	.29	.23	.26	.27	.24	.19	.38	.32	.19	.18	.32	.21	.31	.26	.23	-	.11	.15	.13	.16	.17	.51	.39	.27
BAH2	.41	.41	.48	.38	.36	.36	.37	.37	.33	.20	.34	.33	.25	.27	.29	.29	.24	.43	.35	.21	.22	.37	.23	.34	.28	.26	.10	-	.05	.02	.13	.15	.53	.43	.30
BAH3	.46	.46	.52	.42	.41	.41	.42	.43	.38	.25	.42	.37	.31	.33	.35	.33	.29	.49	.41	.26	.26	.43	.28	.39	.32	.31	.15	.04	-	.08	.16	.17	.61	.48	.35
BAH4	.40	.41	.48	.36	.35	.35	.36	.36	.31	.18	.31	.31	.23	.25	.28	.28	.21	.45	.35	.20	.17	.35	.21	.31	.25	.21	.13	.02	.07	-	.05	.08	.56	.43	.28
BAH5	.44	.44	.49	.40	.38	.39	.38	.39	.33	.22	.36	.33	.27	.29	.32	.32	.26	.46	.38	.25	.22	.39	.25	.33	.29	.25	.15	.12	.14	.05	-	0.0	.54	.44	.32
BAH6	.44	.45	.50	.40	.38	.39	.38	.40	.35	.23	.38	.34	.28	.30	.33	.32	.27	.47	.39	.25	.23	.40	.25	.34	.29	.27	.16	.14	.17	.09	0.0	-	.58	.45	.33
BAH7	.57	.57	.61	.54	.51	.52	.54	.59	.49	.35	.66	.48	.44	.47	.48	.47	.40	.65	.59	.41	.39	.64	.40	.57	.44	.46	.45	.49	.57	.54	.49	.56	-	.65	.51
BER1	.16	.17	.19	.12	.10	.13	.11	.12	.35	.23	.38	.34	.29	.33	.31	.32	.29	.44	.37	.20	.26	.39	.26	.38	.30	.43	.36	.40	.46	.42	.43	.43	.62	-	.11
BER2	.10	.08	.13	.06	.05	.07	.06	.06	.24	.15	.26	.26	.21	.24	.22	.23	.19	.34	.26	.13	.18	.28	.18	.27	.20	.34	.28	.30	.36	.29	.33	.33	.50	.08	-

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CHAPTER 4:

Range-wide population genetic structure of the Caribbean sea fan coral, *Gorgonia ventalina*

ABSTRACT

Generalizations about the population structure of marine species have been difficult to make. In contrast to previous expectations of general panmixus, a growing body of evidence suggests that marine populations can be highly structured over short distances. We describe here the range-wide population genetic structure of an ecologically important Caribbean octocoral, *Gorgonia ventalina*, based on seven polymorphic microsatellite markers. Although patterns of connectivity suggest that *Gorgonia ventalina* populations are connected across hundreds of kilometers, populations are not panmictic and can be differentiated across distances less than one kilometer. Genetic differentiation among localities was positively correlated with geographic distance and negatively correlated with dispersal probability, indicating that distance is an important isolating mechanism for *G. ventalina*, and dispersal via ocean currents is a likely means of connectivity among populations. Across the range, gene diversity was positively correlated with latitude, consistent with a source/sink dynamic driven by ocean currents. High genotypic diversity and absence of duplicate multilocus haplotypes indicate that sex is the primary reproductive mode for *G. ventalina.* A comparative analysis of the population structure of *G. ventalina* and its dinoflagellate symbiont, *Symbiodinium* (previously described in Chapter 3), illustrates that some aspects of population structure are similar between symbiotic partners, yet not completely congruent. These patterns indicate that, while populations of *G. ventalina* and its *Symbiodinium* are likely structured by the same oceanographic forces, their dispersal is not coupled, and symbiont transmission occurs horizontally.

INTRODUCTION

Larval dispersal is the primary means of gene flow for most benthic and demersal marine organisms (Hedgecock 1986). Many species have long-lived, pelagic propagules that can be entrained by ocean currents and disperse across great distances. This high dispersal potential has led to the traditionally held view that marine populations are well connected and demographically open (Jokiel 1984, Palumbi 1992, Caley et al. 1996, Scheltema et al. 1996, Roberts 1997). In general, the amount of time larvae spend in the water column is positively correlated with dispersal distance (Bohonak 1999), and many examples of substantial long distance gene flow do exist (e.g. Rosenblatt and Waples 1986, Shulman and Bermingham 1995, Lessios et al. 2001, Rocha et al. 2002, Neethling et al. 2008). However, exceptions are not uncommon, and a number of recent studies have detected strong population subdivision even within marine species with theoretically high dispersal potential (e.g. Quesada et al. 1995, Planes et al. 1998, Barber et al. 2000, Pritchard et al. 2000, Taylor and Hellberg 2003a) (reviewed in Levin 2006). A number of mechanisms have been proposed to explain such counterintuitive population structure, including spatially differential selection, physical oceanographic barriers (Cowen et al. 2000), or larval behavior/mortality that result in local retention (Burton and Feldman 1982). The relative importance of each of these explanations is a matter of ongoing debate (e.g. Colin 2003, Taylor and Hellberg 2003b, Warner and Palumbi 2003).

Geographic surveys of genetic variation are a particularly useful approach for mapping patterns of connectivity among populations of marine organisms (Hellberg et al. 2002, Thorrold et al. 2002). The small size and broad geographic extent of most planktonic propagules precludes direct estimates of migration by tracking of individuals or mark/release/recapture studies. However, population subdivision and effective population sizes can be inferred from the equilibrium distribution of

selectively neutral diversity, and genetic admixture among differentiated populations provides a signal from which one can estimate timing, rate, direction, and distance of dispersal.

The population genetic structure of a species can also shed light on fundamental aspects of its basic biology and life history. Structured populations have the potential to differentiate and adapt to local selective pressures without the diluting effects of genetic input from disparate regions, whereas panmictic species are more likely to be genetically homogenous throughout their range. Moreover, an accurate understanding of population connectivity is essential for the design of effective conservation and management strategies for threatened species. For example, marine reserve networks, one of the most important tools for managing marine systems, require spatially explicit knowledge of the structure of populations they aim to protect (Palumbi 2003). Protected areas should be large enough to encompass and sustain resident populations and spaced near enough to allow dispersal among reserves (Shanks et al. 2003).

Tropical shallow-water corals are among the marine taxa most threatened by anthropogenic disturbance. In just the past few decades, reefs have suffered massive and accelerating losses worldwide due to numerous threats including climate warming (Hoegh-Guldberg 1999, Hughes et al. 2003), ocean acidification (Hoegh-Guldberg et al. 2007), disease (Harvell et al. 2002), eutrophication (McCook et al. 2001, Szmant 2002), and overfishing (Jackson et al. 2001). A global initiative is underway to protect reef ecosystems through coordinated networks of marine reserves (Kelleher et al. 1995), yet our knowledge of the population structure of most coral species is inadequate to inform management plans (Van Oppen and Gates 2006, Baums 2008). Corals exhibit a diversity of reproductive strategies, ranging from hermaphroditic brooders with crawl-away juveniles to gonochoric broadcast-spawners with positively

buoyant larvae that remain viable for months (Harrison and Wallace 1990). In general, coral population structure tends to correlate with reproductive mode, with brooding corals exhibiting more genetic subdivision than broadcast spawners (e.g. Hellberg 1996, Ayre and Hughes 2000, Nishikawa et al. 2003, Miller and Ayre 2008b, Underwood et al. 2009). However, coral life history characteristics have, in some cases, been found to be poor predictors of genetic structure (Miller and Ayre 2008a), and several studies have shown that population subdivision can exist even in coral species with long pelagic larval duration. For example, the Caribbean coral, *Acropora palmata*, is divided into two large regional subpopulations in the Eastern and Western Caribbean (Baums et al. 2005), and at least five species of broadcasting corals (*Acropora hyacinthus*, *A. cytherea*, *A. millepora*, *A. valida*, and *A. tenuis*) are differentiated across scales less than 10 km on reefs in Australia (Ayre and Hughes 2000, Underwood et al. 2009).

Most tropical shallow-water corals exist as superorganisms – obligate symbioses between a cnidarian host and photosynthetic dinoflagellates of the genus *Symbiodinium*. Photosynthesis by *Symbiodinium* provides the majority of the coral's nutrition (Muscatine 1967, Muscatine et al. 1984) and serves as the predominant source of primary production in the oligotrophic waters of tropical reef ecosystems (Muscatine and Porter 1977). Some corals transmit their *Symbiodinium* vertically from the maternal colony to eggs/larvae. In such cases of tightly coupled dispersal, the population structure of host and symbiont should be highly congruent. More commonly though, coral larvae do not carry with them the *Symbiodinium* of their maternal colony and must acquire symbionts anew each generation (Knowlton and Rohwer 2003). In such cases of horizontal transmission, the dispersal of host and symbiont are independent, adding an important dimension of complexity to the consideration of coral population biology. Yet as with their coral hosts, the population

genetic structure of most *Symbiodinium* lineages is poorly understood. Most of the few published reports of *Symbiodinium* population genetics have found strong local structure on the scale of tens of kilometers or less (Santos et al. 2003, Howells et al. 2009, Kirk et al. 2009), though one study identified substantial connectivity across a much broader scale >200km (Magalon et al. 2006).

Here we describe the range-wide population genetic structure of the common sea fan, *Gorgonia ventalina*, one of the most abundant species of octocoral on Caribbean reefs (Sanchez et al. 1997, Sanchez et al. 1998, Ward et al. 2006). Populations of *G. ventalina* have been heavily impacted over the past two decades by the fungal disease aspergillosis (Guzman and Cortes 1984, Nagelkerken et al. 1997, Kim and Harvell 2004), and numerous studies have investigated the ecology (e.g. Kim et al. 2006), epidemiology (e.g. Jolles et al. 2002), and immunology (e.g. Couch et al. 2008) of this coral/pathogen system. Yet little is known about the population biology, life history, or reproductive biology of *G. ventalina*, or most Caribbean octocorals, for that matter. The only population genetic studies of Caribbean gorgonian corals to date found high levels of differentiation across short distances (<10 km) in *Pseudopterogogia elisabethae* (Gutierrez-Rodriguez and Lasker 2004a, Gutierrez-Rodriguez et al. 2005) and *Briareum asbestinum* (Brazeau and Harvell 1994). However, both *P. elisabethae* and *B. asbestinum* are a surface brooders, and their larvae settle close to the maternal colony (Brazeau and Harvell 1994, Gutierrez-Rodriguez and Lasker 2004b), a relatively uncommon reproductive strategy among Caribbean gorgonians. Observations of spawning or larvae of *G. ventalina* have never been reported, so time of spawning, reproductive mode, and larval duration are unknown. However, *G .ventalina* colonies have never been observed with surface-brooded larvae, and their eggs and polyps are much smaller than those of most brooding corals (C. D. Harvell and L. E. Petes, personal communication) suggesting

that *G. ventalina* is likely a broadcast spawner. As most broadcast spawning corals do not transmit their symbionts vertically (Knowlton and Rohwer 2003), it is also likely that *G.ventalina* larvae acquire *Symbiodinium* from the environment where they settle.

The range-wide population structure of *Symbiodinium* hosted by *G.ventalina* has recently been described (Chapter 3), and here we compare the population structure of the host with those results. Based on the aforementioned predictions regarding the life history of *G. ventalina* and its *Symbiodinium*, we hypothesized that the population structures of these symbiotic partners would be distinct. We expected that populations of *G. ventalina* would be connected across larger distances than its symbiont, reflecting the longer pelagic duration and broader dispersal potential of its propagules. However, given the differentiation observed among populations of other Caribbean organisms with high dispersal potential, we did expect that populations of *G. ventalina* would be regionally structured across its range. Taken together, the present study of the coral host, in conjunction with previous results for its algal symbiont, provides a unique opportunity to explore the population biology of both symbiotic partners as they compose the minimal ecologically functional coral entity.

METHODS

Population Sampling

A total of 1624 samples were collected from 35 sites spanning the range of *G. ventalina* in the Caribbean and Western Atlantic (Figure 4.1, Table 4.1). Sites ranged in depth from 5-24 m, with most around 7 m. Where possible, sites were chosen for consistency in habitat characteristics, namely, hard-bottom fore-reef sites dominated by gorgonian communities. At each site, adult colonies of *G. ventalina* (>30 cm height) were haphazardly sampled along several swimming transects across an area measuring approximately 25 m x 25 m. From each sampled colony, a 4 cm x 4 cm

tissue explant was cut from the apical colony edge and transferred to a labeled bag. This sampling method is known not to cause colony mortality, and most corals regrow the excised tissue rapidly. The number of individuals sampled per site (Table 4.1) varied based on availability, with an average of 46. After collection, samples were transferred to individual 2.0 ml plastic tubes containing a 20% salt-saturated dimethyl sulfoxide (DMSO) solution for preservation (Seutin et al. 1991, Dawson et al. 1998).

DNA Extraction, Amplification, and Genotyping

Whole genomic DNA was extracted from 1 cm^2 tissue subsamples using DNeasy Animal Tissue Kits (Qiagen). Extracted DNA was quantified using a Nanodrop spectrophotometer and diluted to 10 ng/µl with reagent-grade water (Sigma). Each sample was genotyped via polymerase chain reaction (PCR) using primers and run conditions for 10 previously reported microsatellite loci (Andras and Rypien 2009). Because genomic extracts from intact coral colonies contain complex mixtures of DNA from both coral and symbiont, all loci were tested extensively on multiple pure cultures of *Symbiodinium* isolated from *G. ventalina* and other metazoan hosts (Andras et al. 2009) to ensure they did not cross-amplify. PCR products were analyzed on an ABI 3100 Automated Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEMAPPER 3.5 (Applied Biosystems) and validated by eye.

Genetic Diversity

We used the program GENEPOP ON THE WEB (Raymond and Rousset 1995) to calculate observed and expected heterozygosities (Nei 1987, specs) and to test for deviations from Hardy Weinberg equilibrium (HWE). In addition, all loci were tested for evidence of null alleles with the program MICRO-CHECKER (Van

Oosterhout et al. 2004). We assessed linkage disequilibrium among all loci within each locality using the program FSTAT 2.9.3 (Goudet 1995) at a significance level of 0.05 after Bonferroni correction. For each locality, we calculated rarefaction-corrected allelic richness and private allelic richness, scaled to a common sample size of 10 individuals, using the program HP-RARE 1.0 (Kalinowski 2005). The dataset was surveyed for identical multilocus genotypes (clones) using the program GIMLET (Valiere 2002), and significance tests for clonal population structure were performed with GENODIVE 2.0b12 (Meirmans and van Tienderen 2004). This analysis compares the observed diversity of multilocus genotypes to that of permuted datasets, which approximate expectations under random sexual reproduction (Gomez and Carvalho 2000, Stenberg et al. 2003). A significantly lower observed diversity is interpreted as evidence of clonal population structure. Nei's corrected diversity index (Nei 1987) was used as the test statistic with 10000 permutations that randomized alleles within populations.

Population Structure

To investigate the partitioning of genetic diversity among localities and regions, we performed analyses of molecular variance (AMOVA, Excoffier et al. 1992) using the program ARLEQUIN 3.0 (Excoffier et al. 2005). Samples were grouped by collection locality nested within region. Regional designations (Table 4.1) were defined based on the geographic proximity of sampling localities and oceanographic barriers between them. Separate AMOVAs were performed assuming either an infinite allele model (IAM, Kimura and Crow 1964) or a stepwise mutation model (SMM, Ohta and Kimura 1973, Valdes et al. 1993). The SMM assumes that relatedness between two alleles is inversely proportional to their difference in length, and may therefore be a more appropriate model for microsatellites, which are thought

to mutate via the incremental gain or loss of single repeat motifs (Ellegren 2004). To estimate the degree of differentiation among all localities, pairwise measures of F_{st} (Weir and Cockerham 1984), which assumes an IAM, and R_{st} (Slatkin 1995), an F_{st} analog based on a SMM, were also calculated using ARLEQUIN.

We inferred population structure in our data using a Bayesian clustering approach implemented by the program STRUCTURE 2.2.3 (Pritchard et al. 2000, Falush et al. 2003, 2007). This program probabilistically assigns individuals membership among K populations (where K is specified by the user), without regard to collection locality. We employed a model that allowed admixture, assumed correlated allele frequencies among populations, and did not incorporate population origin information. We conducted 20 independent runs at each K from 1-35. Each run consisted of $2x10^6$ Markov Chain Monte Carlo (MCMC) iterations after a burn-in of $2x10^6$ steps. This run-length was sufficient to achieve stabilization of all relevant summary statistics.

In STRUCTURE, the optimal number of genetic clusters is generally selected from the range of tested values of K based on *ad hoc* tests. We examined our results with three different approaches for selecting K. The first is a commonly used method that identifies the highest level of hierarchical population structure by determining the maximum second-order rate of change in posterior probability (ΔK) over a range of K (Evanno et al. 2005). The second method calculates ΔK iteratively for successively parsed datasets to identify clusters at multiple hierarchical scales of population structure (Coulon et al. 2008). The third method, originally proposed by the authors of STRUCTURE, selects the value of K that achieves the highest posterior probability [Pr(X|K)], while maximizing average cluster membership coefficients (Q) (Pritchard et al. 2000). In essence, this approach aims to identify the value of K at which the posterior probability "more or less plateaus" (Pritchard et al. 2007). Ultimately, we

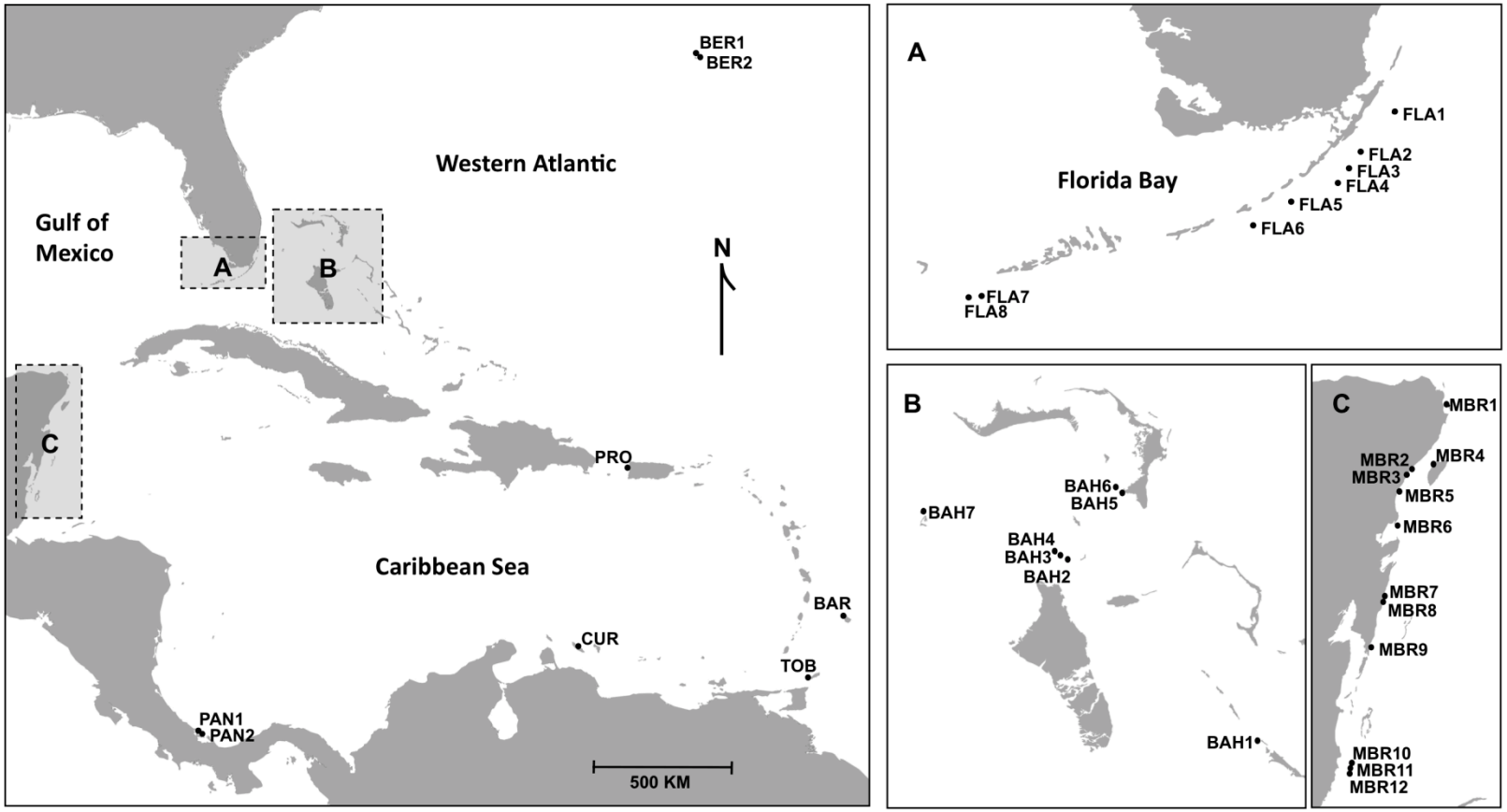

Figure 4.1: Map of 35 localities across the Caribbean Sea and Western Atlantic where *Gorgonia ventalina* colonies were sampled. Regional maps of Florida Keys (**A**), Bahamas (**B**), and Mesoamerican Barrier Reef (**C**) are enlarged.

Table 4.1: Name; abbreviation; depth; GPS coordinates; collection date; sample size (n); rarefaction corrected allelic richness (AR*) and private allelic richness (PAR*) scaled to a common sample size of 10 individuals; expected and observed heterozygosity (He & Ho, respectively); and F_{is} averaged over the seven microsatellite loci included in this study for all 35 sample localities.

Region	Site	Site Code	Depth (m)	GPS	Date Collected	n	AR10	PAR10	He	Ho	Fis
Florida Keys	Florida, Carysfort	FLA1	5	N 25.22°, W 80.21°	8/03	90	6.88	0.05	0.68	0.61	0.08
	Florida, Molasses	FLA2	6	N 25.00°, W 80.37°	8/03	68	6.95	0.07	0.69	0.63	0.07
	Florida, Pickles	FLA3	6	N 24.98°, W 80.41°	8/03	75	6.49	0.10	0.67	0.66	0.02
	Florida, Conch	FLA4	8	N 24.95°, W 80.45°	8/03	47	6.90	0.05	0.68	0.65	0.03
	Florida, Alligator	FLA5	5	N 24.84°, W 80.62°	8/03	68	6.70	0.08	0.67	0.62	0.07
	Florida, Tennessee	FLA6	6	N 24.74°, W 80.78°	8/03	83	6.96	0.10	0.69	0.65	0.05
	Florida, Western Dry Rocks	FLA7	6	N 24.44°, W 81.92°	8/03	35	6.64	0.14	0.65	0.62	0.05
	Florida, Sand Key	FLA8	5	N 24.45°, W 81.87°	8/03	33	6.95	0.12	0.69	0.68	-0.01
Mesoamerican Barrier Reef	Mexico, Isla Mujeres, Tavos	MBR1	9	N 21.24°, W 86.73°	4/03	47	7.00	0.07	0.70	0.67	0.02
	Mexico, Akumal, Media Luna	MBR2	9	N 20.40°, W 87.30°	4/03	68	6.88	0.09	0.69	0.65	0.05
	Mexico, Akumal, South Point	MBR3	9	N 20.38°, W 87.31°	4/03	12	5.57	0.04	0.62	0.64	-0.06
	Mexico, Cozumel, Paradise	MBR4	8	N 20.51°, W 86.94°	4/03	69	6.80	0.11	0.70	0.69	0.01
	Mexico, Tulum, La Piscina	MBR5	12	N 20.22°, W 87.74°	5/03	52	6.74	0.03	0.68	0.62	0.10
	Mexico, Punta Allen	MBR6	9-12	N 19.78°, W 87.43°	7/06	36	6.98	0.11	0.67	0.63	0.09
	Mexico, Mahahual, El Jardin	MBR7	17	N 18.85°, W 87.63°	5/03	42	7.14	0.06	0.69	0.65	0.06
	Mexico, Mahahual, Site 2	MBR8	8	N 18.84°, W 87.64°	5/03	53	6.61	0.10	0.69	0.65	0.04
	Mexico, Xcalak, Dona Nica	MBR9	11	N 18.29°, W 87.81°	5/03	50	7.35	0.16	0.71	0.64	0.09
	Belize, Southwater Caye, Long Reef	MBR10	5	N 16.77°, W 88.08°	5/03	51	7.04	0.08	0.69	0.65	0.05
	Belize, Carrie Bow Caye	MBR11	5	N 16.80°, W 88.08°	5/03	24	7.05	0.08	0.69	0.65	0.04
	Belize, Southwater Caye, East Side	MBR12	9	N 16.81°, W 88.07°	5/03	51	7.35	0.06	0.71	0.62	0.11
Panama	Panama, Bocas del Toro, Punta Vieja	PAN1	5-8	N 9.260°, W 82.12°	10/05	34	5.93	0.06	0.62	0.57	0.07
	Panama, Bocas del Toro, Hospital Point	PAN2	5-8	N 9.333°, W 82.21°	10/05	20	5.62	0.00	0.60	0.60	0.00
Curacao	Curacao	CUR	9-12	N 12.11°, W 68.97°	7/06	58	5.87	0.11	0.65	0.59	0.11
Tobago	Tobago	TOB	9-12	N 11.19°, W 60.79°	4/08	53	4.98	0.00	0.58	0.58	-0.01
Barbados	Barbados	BAR	9-12	N 13.17°, W 59.64°	9/06	74	6.31	0.08	0.62	0.56	0.09
Puerto Rico	Puerto Rico, Steps Beach, Rincon	PRO	5-6	N 18.35°, W 67.26°	8/06	62	6.90	0.02	0.69	0.65	0.04
Bahamas	Bahamas, Lee Stocking Island	BAH1	5-6	N 23.78°, W 76.13°	5/03	33	6.42	0.01	0.67	0.60	0.09
	Bahamas, Chub Cay	BAH2	9-12	N 25.40°, W 77.92°	5/05	25	6.79	0.06	0.66	0.63	0.04
	Bahamas, GPS121	BAH3	11-12	N 25.42°, W 77.98°	5/04	19	6.52	0.01	0.68	0.73	-0.10
	Bahamas, Rum Cay	BAH4	12-15	N 25.45°, W 78.03°	5/04	12	6.57	0.00	0.73	0.74	-0.04
	Bahamas, Sandy Point	BAH5	8-9	N 25.99°, W 77.42°	5/04	25	6.98	0.10	0.71	0.66	0.07
	Bahamas, Gorda Rock	BAH6	5-6	N 26.04°, W 77.47°	5/04	22	6.38	0.06	0.65	0.64	0.02
	Bahamas, Bimini	BAH7	21-24	N 25.81°, W 79.28°	5/05	17	6.73	0.02	0.68	0.58	0.15
Bermuda	Bermuda, Castle Harbour	BER1	5-6	N 32.33°, W 64.67°	5/07	55	6.16	0.00	0.69	0.65	0.06
	Bermuda, Crescent	BER2	5-6	N 32.39°, W 64.79°	5/07	61	6.35	0.00	0.69	0.67	0.02

chose to use this final approach, since the two methods based on ΔK produced estimates of K that were either too low or difficult to interpret. Moreover, simulation studies have found that the method suggested by Pritchard *et al*. (2000) performs as well or better than the methods based on ΔK (Waples and Gaggiotti 2006). For the chosen value of K, cluster membership coefficients for the10 runs with the highest Pr(X|K) were matched and averaged by the program CLUMPP (Jakobsson and Rosenberg 2007), using the Greedy algorithm with $1x10^5$ random input orders.

In addition to Bayesian clustering, we also constructed an unrooted dendrogram based on Nei's unbiased genetic distance (Nei 1978) to visualize relationships between localities. The dendrogram was generated with the unweighted pair group method with arithmetic mean (UPGMA) using the program TOOLS FOR POPULATION GENETICS ANALYSIS (TFPGA) 1.3 (Miller 1997).

Estimation of Migration Rates

We assessed migration among localities and regions using two complimentary approaches. To estimate contemporary migration (i.e. within the past several generations) we used the program BAYESASS 1.3 (Wilson and Rannala 2003). This program employs a Bayesian MCMC approach to identify migrants or recent descendants of migrants based on transient linkage disequilibrium among multilocus genotypes from different source populations. Because BAYESASS accommodates a maximum of only 25 populations, we were unable to estimate pairwise migration rates among all localities. Instead, localities were grouped to estimate migration rates among regions (designated in Table 4.1). We performed $21x10^6$ iterations, with a burnin of $2x10^6$ and a sampling frequency of 2000. This run length was sufficient for the posterior probability to achieve convergence. The "delta values" for allele frequency, level of inbreeding, and migration rate, which define the maximum amount

each parameter can change after each iteration, were adjusted to 0.06, 0.08, and 0.25, respectively. These values resulted in MCMC acceptance ratios between 40-60%, which tend to maximize log likelihood values (Wilson and Rannala 2003).

To compliment our analysis with BAYESASS, we estimated long-term migration ($M = m/\mu$ = migration rate/mutation rate) and effective population sizes ($\theta = 4*N_e*\mu = 4*$effective population size*mutation rate) among regions using the program MIGRATE 3.0 (Beerli and Felsenstein 2001). As MIGRATE employs a coalescent approach, it estimates dispersal over long-term evolutionary history, whereas BAYESASS estimates migration over short-term ecological history (Faubet et al. 2007). We implemented a maximum likelihood search strategy with a continuous Brownian motion model of microsatellite mutation. We used a UPGMA tree as our starting genealogy with initial estimates of M and θ based on F_{st}. Our first run consisted of 10 short chains ($1x10^5$ trees sampled, 10000 recorded), 6 long chains ($5x10^5$ trees sampled, 50000 discarded as burn-in, 50000 recorded), and a static heating scheme of four temperatures (1.0, 1.5, 3.0, 10000). This run length was sufficient to achieve convergence of the 99.5% likelihood percentile. We then used the estimates of M and θ from the initial run as starting parameters for a second run of equal length to ensure consistency across runs.

Spatial and Oceanographic Correlation Analyses

To infer mechanisms that may be responsible for the observed patterns of differentiation/connectivity, we compared our estimates of genetic structure and dispersal to geographic distances and modeled oceanographic connectivity among localities. Estimates of oceanographic connectivity were derived from simulations reported by Galindo *et al*. (2006) and used with the authors' permission. These simulations, implemented by the Miami Isopycnic Coodinate Ocean Model (MICOM),

use historical wind data to estimate near-surface ocean currents. Specifically, Galindo *et al*. (2006) estimated dispersal among 87 randomly chosen localities across the Caribbean by tracking the movements of simulated passively drifting particles. At the end of each simulation, the proportion of particles released at one site that arrive at another (including 50 km buffer zones around all sites), represents the probability of passive directional dispersal between those localities. Simulations were repeated in the winter (Julian days 15-57), spring (Julian days 135-177), and summer (Julian days 205-247) of each year from 1982-1986 (see Galindo et al. 2006 for full details). For our analyses, we selected locations from the model that were closest to our sampling localities and compared the corresponding dispersal probabilities averaged across all five modeled years to our estimates of genetic differentiation and migration. Localities in Bermuda were omitted, as they were not included in the simulation study. We performed Mantel tests (Mantel 1967) with the program ARLEQUIN 3.0 (Excoffier et al. 2005) to evaluate the correspondence between pairwise matrices of modeled dispersal probability and genetic differentiation (Fst & Rst), with 1000 random permutations to assess significance. Because dispersal probabilities were bidirectional and asymmetric, we averaged dispersal in both directions [i.e. (A→B + B→A)/2] to compare them with our estimates of Fst & Rst, which are symmetric. We also ran separate Mantel tests to compare our estimates of migration among regions to the averaged dispersal probabilities from all model locations that were located within our regional designations. This test was performed with the program CADM (congruence among distance matrices; Legendre 2001), which accommodates Mantel tests of full asymmetric matrices.

To evaluate the relationship between genetic differentiation and geographic distance, we performed Mantel tests (ARLEQUIN 3.0; 1000 random permutations) of genetic differentiation (F_{st} & R_{st}) versus Euclidian (straight-line geographic) distances

among localities. We also performed tests of spatial genetic autocorrelation to evaluate whether the degree of genetic similarity between individuals is correlated with the geographic distance between them. Unlike Mantel tests, which identify strong patterns of differentiation extending over the full geographic sample range, spatial autocorrelation analyses allow for the independent consideration of comparisons within different distance classes, thereby providing greater resolution of spatial genetic structure (Clark and Richardson 2002, Epperson 2005). We used the program GENALEX 6.1 (Peakall and Smouse 2006) to evaluate spatial autocorrelation in our dataset following two alternative approaches, one using *fixed distance classes* of the same size, and another using *multiple distance classes* of increasing size. All analyses were based on pairwise genetic distances among all individuals which were calculated as described by Peakall *et al.* (1995). For the *fixed distance class* analysis, we measured the autocorrelation coefficient of genetic distance (r) within geographic distance classes of a fixed size (50 km, 62 total distance classes). The behavior of r over increasing distance can illustrate the shifting balance between gene flow and genetic drift. In cases where positive spatial structure is present, r will decrease with increasing distance size class, and the point at which r first becomes zero (i.e. the x-intercept of the autocorrelogram) provides an estimate of the approximate extent of positive spatial genetic structure (Sokal and Wartenberg 1983, Smouse and Peakall 1999, Peakall et al. 2003, Epperson 2005). Since the x-intercept of r has been shown to depend on the size of the fixed distance class employed (Peakall et al. 2003), we also implemented an alternative approach that is less sensitive to distance class size. In this *multiple distance class* approach, r was calculated for 32 geographic distance classes of increasing size, ranging from 100 km to 3200, the maximum distance between sampling localities. For all spatial autocorrelation analyses, significance was

assessed relative to 1000 random permutations of the dataset, and 95% confidence intervals of *r* were generated from 1000 bootstrap replicates.

RESULTS

Genetic Diversity

Of the ten microsatellite loci developed for this study, three (SYM203, GV31, GVc9) showed significant deviations from Hardy-Weinberg equilibrium at nearly all sampling localities and appeared to suffer from substantial null alleles, as evidenced by a significant excess of homozygotes. Consequently, all results reported here exclude these loci (although their inclusion did not alter the results substantially - data not shown). The remaining seven loci did not deviate significantly from Hardy-Weinberg or linkage equilibrium within localities.

There were no clonal multilocus genotypes detected throughout the entire dataset, and no significant evidence of clonal population structure. Mean gene diversity (H_e) and allelic richness (AR_{10} - corrected to a sample of 10 individuals) for each locality were high (H_e=0.58-0.74, AR_{10} = 4.98-7.35, Table 4.1), and were positively correlated with latitude (H_e: R^2=0.303, p=0.0006; AR_{10}: R^2=0.148, p=0.0224; Figure 4.2).

Population Structure

AMOVAs based on both an IAM and SMM detected significant differentiation among regions and populations within regions (Table 4.2). Pairwise F_{st} comparisons between localities (Table 4.3, below diagonal) ranged from 0.010 to 0.192 (overall F_{st} = 0.077), and nearly all were significant (98.5%). Estimates of R_{st} (Table 4.3, above diagonal) were higher overall (overall R_{st} = 0.16; range = 0.010 to 0.550;), although fewer pairwise comparisons were significant (84.7%).

For the Bayesian clustering analyses run with STRUCTURE, the posterior probability [Pr(X|K)] rose sharply over K 1-5, and settled into a more gradual yet consistent ascent thereafter (Figure 4.3). In such cases, where the model choice criterion increases indefinitely with increasing K, the authors of STRUCTURE caution against overestimating K, and advise choosing the smallest value of K that resolves the major structure in the data (Pritchard et al. 2000, Pritchard et al. 2007). Based on this interpretive criterion, we chose a seven-population model (K=7) as the best solution for the Bayesian clustering analysis, as values of K<7 failed to capture certain features of biogeographically sensible structure, and values of K>7 reduced average membership values and increased apparent admixture among clusters.

In the K=7 solution, a total of 1250 individuals (77%) were assigned to one of the seven clusters with a probability of at least 0.6. The clusters were generally distributed cohesively across contiguous or adjacent localities and identify five broad genetically differentiated regions (Figure 4.4):

1. The large majority of individuals from the two **Bermuda** localities were assigned to the Yellow Cluster, which is relatively uncommon across the rest of the range.
2. Localities in the **Florida Keys** were dominated by either the Orange Cluster or Red Cluster.
3. The Brown and Green Clusters were almost exclusively limited to localities from **the Mesoamerican Barrier Reef and Panama**,
4. The Purple Cluster was restricted to **the Windward Islands of Barbados and Tobago** in the East.
5. Most individuals from **the Bahamas** were assigned to the Blue Cluster, although members of the Yellow and Red Clusters are also prevalent among the Bahamas localities, indicating some connectivity with Bermuda and the Florida Keys.

Interestingly, the majority of individuals from **Curacao** were also assigned to the Blue Cluster.

Many of the patterns observed in the Bayesian clustering analyses were also evident in the UPGMA dendrogram of genetic distances (Figure 4.5). Most localities from the same region tended to form exclusive or nearly exclusive groups that correspond well with the broad regional populations identified by STRUCTURE. Namely, the Florida Keys comprised their own exclusive group, as did Bermuda, the Bahamas, and most localities along the Mesoamerican Barrier Reef.

Estimation of Dispersal

Based on rates of recent migration estimated by the program BAYEASS, eight of the nine regions examined in this study (defined in Table 4.1) are almost entirely self-recruiting (i.e. >99%). Only Panama was an exception, with an estimated 26% of individuals identified as migrants or recent descendants of migrants from the Mesoamerican Barrier Reef. In contrast, all of the maximum likelihood estimates of long-term migration calculated with MIGRATE were greater than zero (Table 4.4), and 75% of pairwise bidirectional migration rates were asymmetric based on their non-overlapping 95% confidence intervals. Certain migration patterns were consistent throughout regions. For example, the Florida Keys and the Mesoamerican Barrier Reef were definitively identified as sink populations, as all rates of immigration into these regions were significantly greater than rates of emigration. Correspondingly, estimated populations sizes (θ, Table 4.4) were substantially larger for Florida and the Mesoamerican Barrier Reef than all other regions. Elsewhere source/sink dynamics were more variable among regional comparisons, and, in some cases were inconsistent with expectations based on geography and prevailing currents. For example, multiple comparisons identify Tobago as a net sink population, although it is situated in the far

southeastern Caribbean, oceanographically "upstream" of nearly all other sites, while other comparisons identify Bermuda, the farthest "downstream" locality, as a net source.

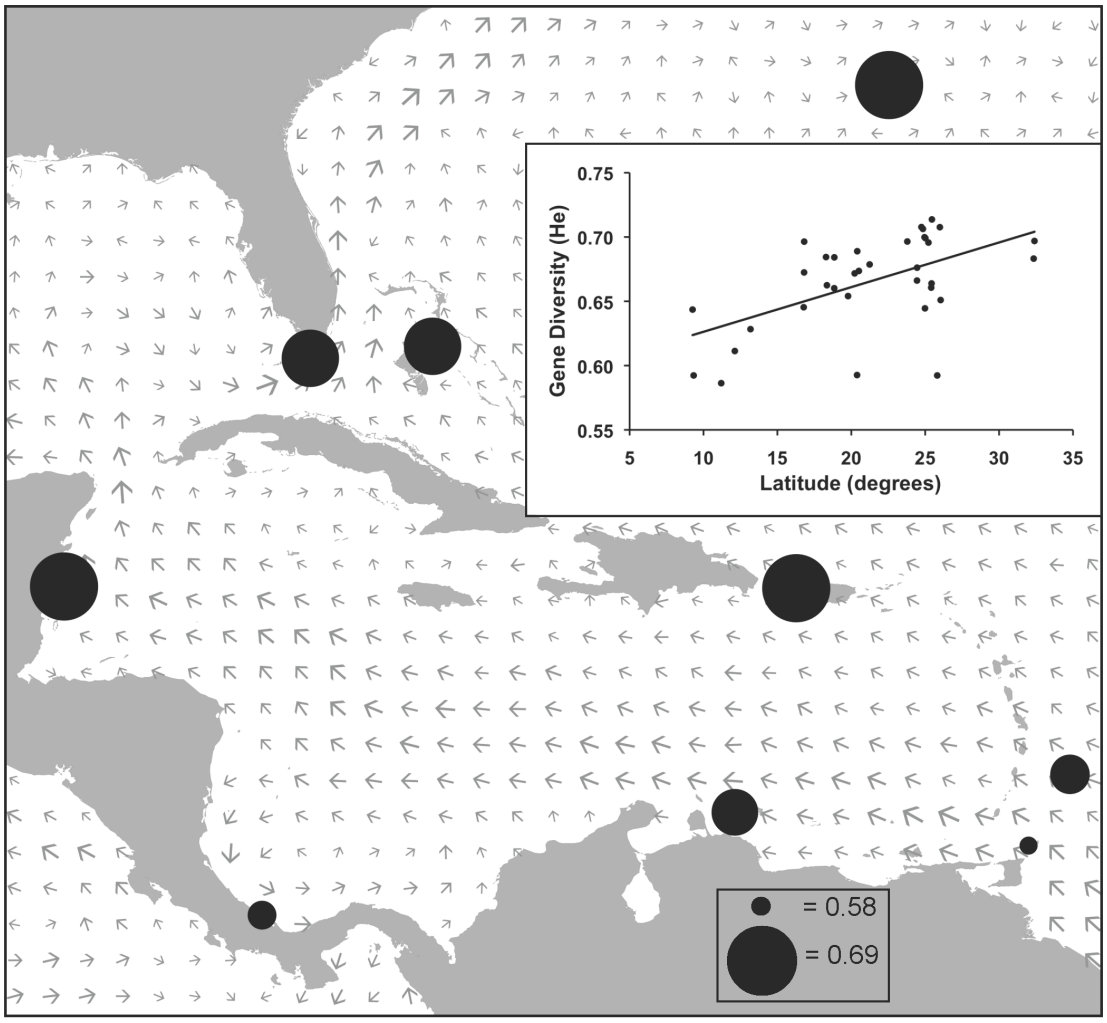

Figure 4.2: Geographic distribution of genetic diversity among *G. ventalina* populations. Diameter of filled circles on map is scaled to the gene diversity (expected heterozygosity) of each sampling region. Inset plot depicts linear regression of gene diversity versus latitude (R^2=0.303, p=0.0006). Arrows on map illustrate average annual ocean currents, oriented to direction scaled to relative velocity, at 1x1 degree intervals (Ocean current data used with permission from Mariano et al. 1995).

Table 4.2: Results from AMOVAs implementing either an infinite allele model (**A**) or a stepwise mutation model (**B**).

	Model Used	Source of Variation	Degrees of Freedom	Sum of Squares	Variance Component	Percent Variation	Fixation Indices
A)	IAM	Among regions	8	498.91	Va = 0.157	4.31	F_{CT} = 0.043*
		Among localities within regions	26	385.10	Vb = 0.122	3.36	F_{SC} = 0.035*
		Among individuals within a locality	1572	6297.06	Vc = 3.001	17.97	F_{ST} = 0.077*
		Within Individuals	1607	4340.00	Vd=2.701	74.36	
B)	SMM	Among regions	8	68102.65	Va = 24.887	11.24	R_{CT} =0.112*
		Among localities within regions	26	27116.21	Vb = 9.326	4.21	R_{SC} =0.047*
		Among individuals within a locality	1572	342263.16	Vc = 30.614	13.83	R_{ST} =0.155*
		Within Individuals	1607	251491.50	Vd=156.497	70.71	

* = $P < 0.001$

Table 4.3: Pairwise Fst values (below diagonal) and Rst values (above diagonal) for all loci. Site codes are given in Table 4.1. p<0.05=*; p<0.00001=**; Non-significant values are shaded gray.

SITE	FLA1	FLA2	FLA3	FLA4	FLA5	FLA6	FLA7	FLA8	MBR1	MBR2	MBR3	MBR4	MBR5	MBR6	MBR7	MBR8	MBR9	MBR10	MBR11	MBR12	PAN1	PAN2	CUR	TOB	BAR	PRO	BAH1	BAH2	BAH3	BAH4	BAH5	BAH6	BAH7	BER1	BER2
FLA1	-	.03	.05	0	0	0	.02	.03	.19	.08	.11	.19	.05	.03	.06	.18	.06	.33	.27	.12	.09	.28	.03	.08	.07	.12	.06	0	.03	0	0	0	0	.18	.13
FLA2	.01	-	0	.01	.03	.06	.01	.01	.08	.01	.04	.09	.06	.01	.01	.07	.01	.19	.14	.04	.11	.17	.04	.16	.14	.22	.08	.05	.10	.05	.07	.06	.03	.27	.15
FLA3	.03	.01	-	.03	.05	.09	.01	.01	.09	.00	.03	.10	.09	.02	.02	.06	.02	.20	.15	.04	.11	.19	.07	.21	.19	.26	.11	.06	.13	.09	.11	.08	.06	.32	.18
FLA4	.01	.00	.03	-	0	.01	.02	.03	.15	.05	.09	.16	.05	.02	.03	.14	.02	.28	.22	.08	.13	.26	.01	.09	.07	.13	.09	0	.05	0	.02	.02	0	.19	.10
FLA5	.00	.01	.03	.00	-	0	.02	.04	.20	.09	.13	.21	.07	.04	.06	.19	.06	.33	.27	.13	.13	.31	.02	.06	.05	.10	.10	0	.05	0	0	.01	0	.16	.10
FLA6	.01	.02	.06	.01	.00	-	.04	.06	.24	.12	.16	.24	.07	.06	.09	.22	.09	.37	.31	.17	.12	.32	.04	.05	.04	.07	.08	.01	.03	0	0	0	0	.13	.11
FLA7	.01	.02	.03	.02	.02	.03	-	0	.12	.03	.02	.13	.07	.03	.04	.09	.04	.25	.19	.07	.05	.21	.06	.13	.13	.19	.06	.02	.07	.04	.05	.02	.02	.27	.19
FLA8	.02	.02	.04	.02	.02	.03	.02	-	.12	.03	.01	.13	.09	.02	.04	.09	.05	.25	.19	.08	.03	.21	.07	.14	.14	.21	.07	.03	.09	.07	.06	.02	.04	.28	.20
MBR1	.07	.08	.11	.06	.06	.06	.07	.09	-	.03	.04	0	.14	.07	.04	0	.05	.01	0	.01	.18	.04	.13	.32	.29	.39	.17	.17	.22	.20	.22	.21	.17	.42	.27
MBR2	.04	.05	.08	.04	.03	.03	.06	.06	.02	-	0	.05	.09	.02	.01	.02	.01	.12	.07	.01	.10	.12	.06	.22	.20	.28	.12	.07	.14	.10	.13	.10	.07	.31	.18
MBR3	.09	.09	.15	.07	.07	.07	.09	.12	.10	.06	-	.05	.13	.03	.04	.01	.05	.15	.10	.03	.05	.13	.09	.24	.22	.34	.12	.08	.17	.19	.16	.10	.11	.41	.29
MBR4	.07	.08	.11	.06	.06	.05	.09	.08	.05	.03	.08	-	.12	.06	.05	.02	.07	.04	.03	.01	.16	.01	.15	.34	.30	.40	.14	.18	.20	.20	.23	.21	.18	.42	.29
MBR5	.04	.05	.09	.03	.03	.03	.05	.07	.04	.01	.04	.04	-	.03	.04	.16	.05	.26	.22	.08	.12	.18	.05	.19	.12	.22	.02	.06	.01	.02	.05	.07	.04	.27	.19
MBR6	.07	.08	.12	.06	.06	.05	.08	.09	.06	.02	.02	.04	.01	-	0	.07	.01	.17	.11	.03	.07	.12	.02	.13	.12	.21	.05	.02	.06	.03	.05	.04	.02	.20	.09
MBR7	.06	.05	.09	.04	.04	.04	.07	.06	.06	.01	.05	.03	.02	.01	-	.05	0	.14	.09	.01	.11	.12	.03	.17	.15	.25	.09	.04	.09	.06	.08	.08	.04	.25	.11
MBR8	.07	.07	.10	.07	.06	.06	.08	.08	.05	.03	.12	.07	.06	.08	.06	-	.06	.05	.03	.02	.15	.05	.14	.31	.29	.39	.17	.17	.23	.21	.22	.20	.18	.43	.29
MBR9	.05	.06	.10	.04	.04	.04	.06	.07	.02	.00	.06	.04	.01	.02	.02	.04	-	.15	.09	0	.15	.15	.02	.19	.14	.24	.11	.05	.10	.06	.09	.09	.04	.28	.14
MBR10	.06	.06	.09	.07	.06	.07	.07	.08	.07	.08	.14	.09	.09	.13	.10	.07	.09	-	0	.08	.31	.05	.23	.44	.40	.52	.30	.31	.36	.36	.37	.35	.32	.54	.40
MBR11	.05	.05	.09	.04	.03	.05	.05	.07	.04	.04	.07	.03	.04	.06	.04	.06	.05	.02	-	.04	.28	.07	.16	.38	.33	.47	.27	.24	.30	.29	.30	.28	.24	.49	.33
MBR12	.04	.04	.09	.03	.03	.03	.05	.05	.02	.00	.05	.02	.01	.02	.01	.02	.00	.06	.02	-	.15	.07	.07	.27	.22	.33	.13	.11	.15	.14	.16	.15	.11	.38	.23
PAN1	.06	.07	.11	.06	.05	.04	.08	.07	.07	.03	.05	.04	.03	.02	.02	.06	.03	.12	.05	.03	-	.22	.14	.20	.21	.28	.05	.08	.11	.13	.12	.06	.11	.35	.30
PAN2	.06	.06	.10	.06	.05	.07	.06	.08	.09	.06	.11	.09	.06	.10	.08	.07	.07	.06	.05	.05	.08	-	.21	.41	.35	.50	.19	.27	.28	.34	.31	.29	.29	.53	.41
CUR	.08	.07	.11	.06	.06	.08	.07	.11	.12	.10	.08	.12	.07	.11	.10	.12	.10	.08	.06	.08	.10	.07	-	.08	.04	.11	.11	.01	.05	0	.03	.04	0	.12	.04
TOB	.08	.09	.12	.08	.07	.07	.09	.09	.10	.07	.12	.09	.08	.09	.07	.11	.08	.15	.09	.07	.08	.11	.15	-	.03	.05	.19	.06	.11	.06	.04	.05	.06	.03	.09
BAR	.09	.09	.13	.08	.08	.07	.10	.12	.10	.05	.05	.07	.05	.03	.05	.11	.06	.15	.08	.06	.04	.12	.12	.10	-	.01	.15	.04	.05	.01	.01	.04	.02	.06	.09
PRO	.06	.05	.09	.05	.04	.05	.07	.09	.10	.08	.10	.10	.07	.10	.09	.10	.08	.07	.06	.06	.10	.07	.05	.12	.11	-	.24	0.1	.10	.07	.05	.07	.08	.12	.21
BAH1	.03	.03	.07	.03	.02	.02	.04	.04	.07	.04	.06	.05	.03	.05	.03	.06	.04	.07	.04	.03	.02	.05	.07	.09	.07	.05	-	.08	.02	.06	.07	.06	.08	.30	.25
BAH2	.04	.04	.09	.04	.03	.04	.05	.06	.10	.06	.08	.08	.04	.06	.04	.09	.06	.07	.05	.05	.05	.06	.07	.10	.07	.05	.02	-	.02	0	0	0	0	.15	.11
BAH3	.06	.06	.10	.06	.05	.06	.06	.08	.11	.07	.09	.09	.06	.08	.07	.11	.08	.07	.05	.07	.07	.06	.06	.12	.10	.06	.03	.02	-	0	0	.01	.01	.20	.19
BAH4	.05	.05	.09	.05	.04	.04	.06	.07	.09	.06	.11	.07	.06	.08	.05	.09	.06	.07	.05	.05	.06	.07	.08	.08	.10	.07	.03	.01	.01	-	0	0	0	.15	.12
BAH5	.03	.04	.08	.03	.02	.03	.04	.05	.08	.05	.08	.06	.04	.07	.04	.08	.05	.06	.03	.04	.05	.04	.07	.07	.09	.06	.02	.02	0	0	-	0	0	.11	.11
BAH6	.05	.05	.10	.04	.03	.04	.06	.07	.11	.06	.09	.08	.05	.07	.05	.11	.07	.07	.05	.06	.06	.05	.07	.11	.09	.06	.03	.00	.00	.01	.01	-	0	.16	.15
BAH7	.11	.13	.19	.11	.11	.09	.16	.16	.15	.09	.14	.12	.11	.09	.10	.15	.11	.17	.14	.11	.11	.17	.16	.18	.13	.15	.11	.12	.12	.08	.11	.09	-	.16	.10
BER1	.06	.07	.10	.06	.05	.05	.07	.09	.10	.08	.11	.10	.08	.10	.09	.10	.08	.10	.08	.08	.08	.09	.10	.09	.11	.08	.05	.05	.07	.05	.04	.07	.15	-	.09
BER2	.04	.04	.08	.05	.04	.04	.06	.06	.08	.06	.10	.09	.07	.09	.06	.07	.06	.08	.06	.06	.06	.07	.09	.09	.10	.07	.03	.05	.07	.06	.05	.07	.15	.02	-

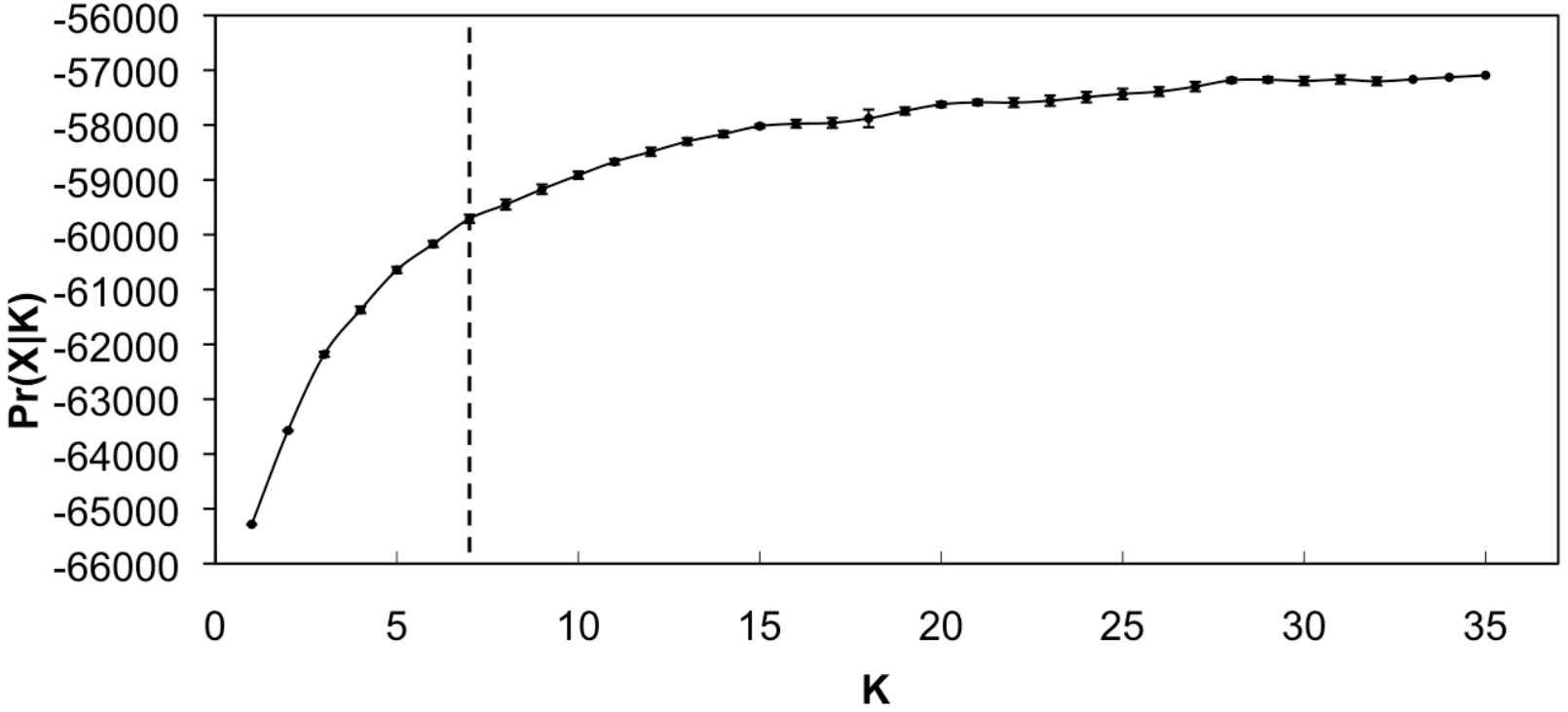

Figure 4.3: Posterior probability [Pr(X|K)] of Bayesian clustering analyses of *G. ventalina* for each value of K 1-35. The vertical dashed line marks K=7, chosen as the optimal number of genetic clusters.

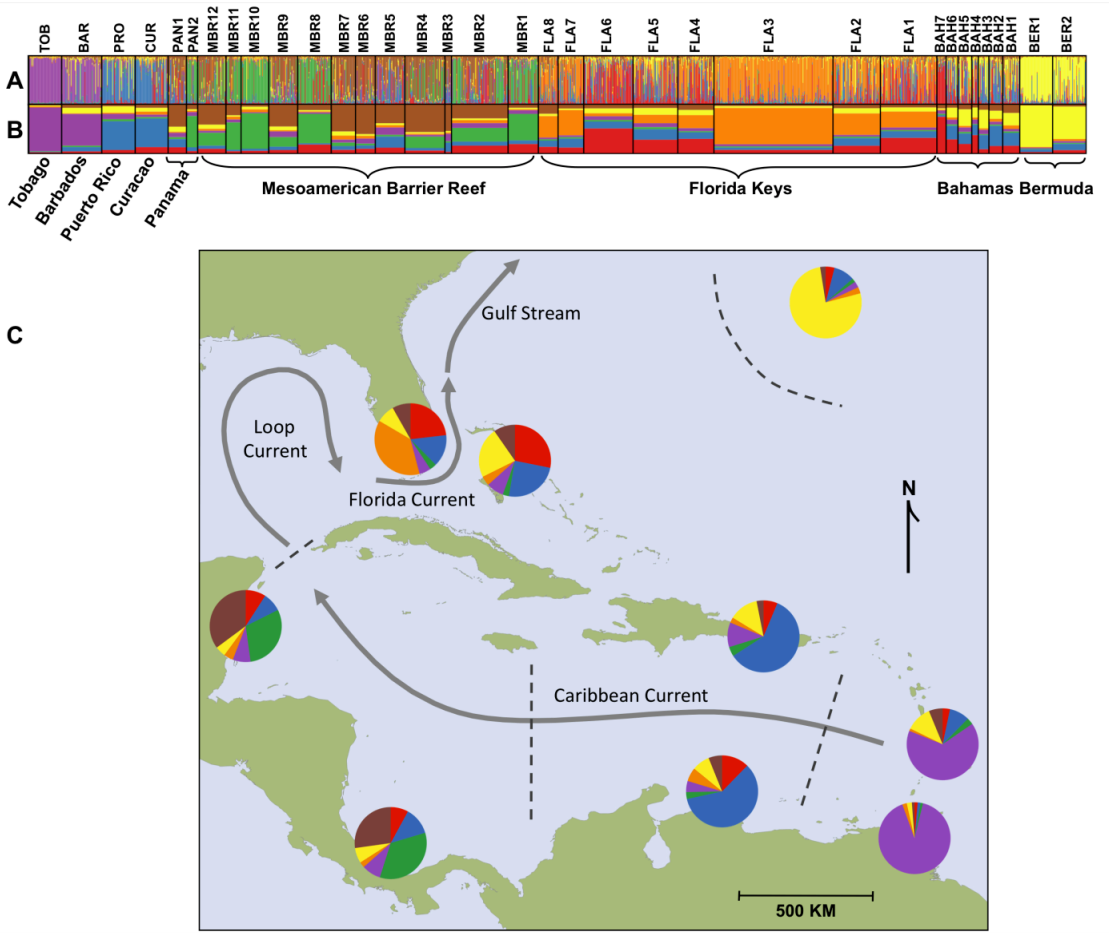

Figure 4.4: Bayesian population assignment of *G. ventalina* as determined by the program STRUCTURE. Samples were assigned among seven color-coded genetic clusters, (Red, Orange, Yellow, Green, Blue, Purple, Brown). **A**. Cluster assignments of individual samples, where each thin vertical line represents a single *Gorgonia ventalina* colony. **B**. Average cluster assignments for each locality. Individuals are grouped by locality (labels above) and region (labels below). Localities are arrayed roughly along the trajectory of predominant ocean currents, moving from the Windward Islands in the east, westward through the Caribbean basin, and north through the Florida Keys Bahamas, and Bermuda. Locality codes are given in Table 4.1. **C**. Cluster assignments are averaged for each region and displayed as pie charts. Arrows denote major current patterns. Dashed lines denote divisions between regional populations (placement not exact). See text for detail.

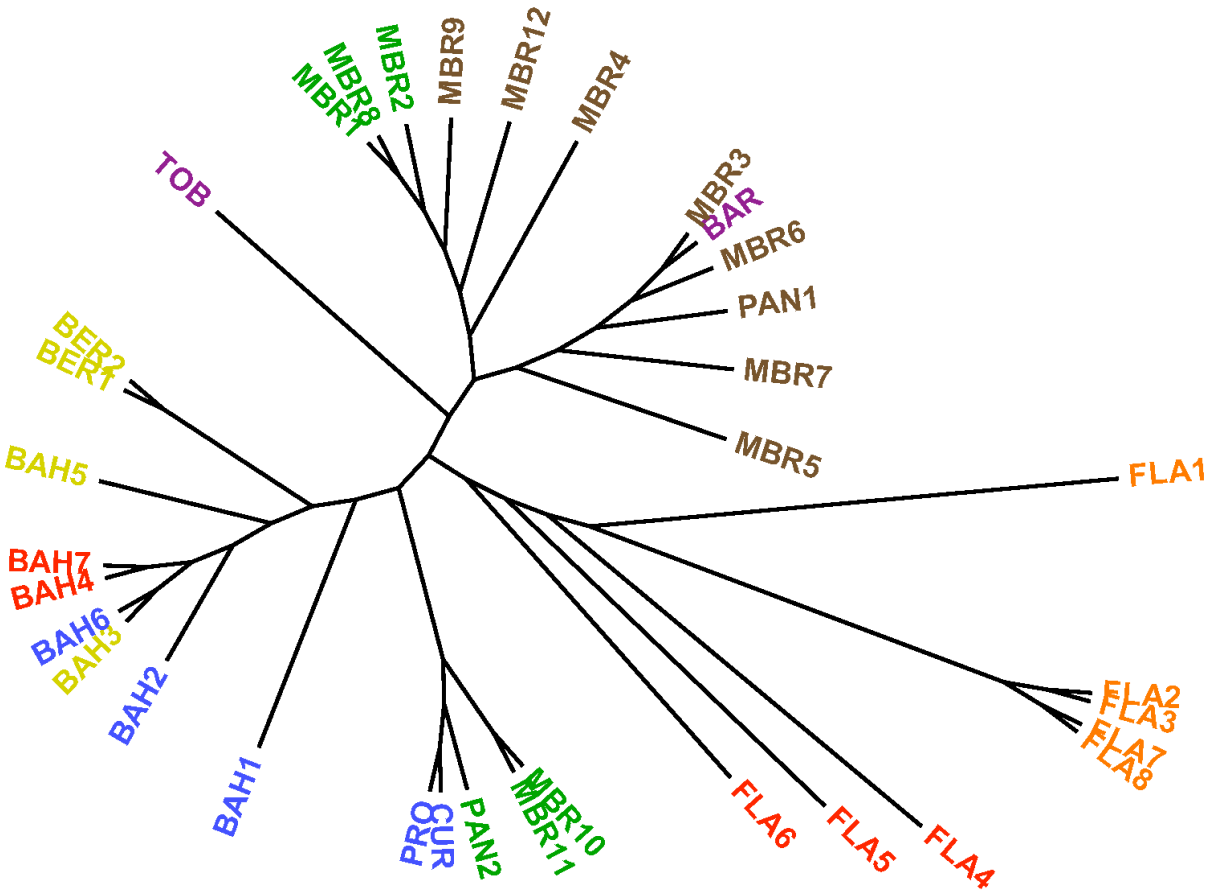

Figure 4.5: UPGMA cladogram of Nei's unbiased genetic distance, depicting relationships among *Gorgonia ventalina* at 35 localities in the Caribbean and Western Atlantic. Localities are color-coded according to their dominant Bayesian cluster assignment (see Figure 4.4). Locality codes are given in Table 4.1.

Table 4.4: Estimated long-term migration rates (**above and below diagonal**: $M = m/\mu$ = migration rate/mutation rate) and effective population size (**along diagonal**: $\theta = 4*N_e*\mu = 4*$effective population size*mutation rate) for regional populations of *G. ventalina*. Source populations are given in rows; Recipient populations are given in columns. All estimates are listed as 95% confidence ranges about the maximum likelihood value. In cases where bidirectional migration estimates are asymmetric (i.e. 95% confidence ranges do not overlap), the net source population is shaded blue and the net sink population is shaded pink. Bidirectional migration estimates with overlapping 95% confidence ranges are shaded gray.

		Recipient Population								
		Florida Keys	**Mesoamerican Barrier Reef**	**Panama**	**Curacao**	**Tobago**	**Barbados**	**Puerto Rico**	**Bahamas**	**Bermuda**
Source Population	**Florida Keys**	1.39-1.44	7.45-8.13	1.26-1.55	1.39-1.70	0.94-1.19	0.52-0.72	1.01-1.28	1.85-2.20	1.69-2.03
	Mesoamerican Barrier Reef	8.42-9.16	1.51-1.57	1.20-1.48	1.14-1.41	0.83-1.07	1.11-1.38	0.98-1.24	2.39-2.78	2.19-2.57
	Panama	1.66-2.18	2.21-2.79	0.74-0.82	0.87-1.25	0.84-1.21	0.53-0.83	0.92-1.31	2.07-2.64	1.27-1.72
	Curacao	2.04-2.57	2.58-3.18	0.31-0.53	0.75-0.83	1.00-1.39	1.20-1.62	0.55-0.85	1.56-2.03	1.11-1.52
	Tobago	1.36-1.77	1.66-2.11	0.49-0.75	0.72-1.03	0.90-0.99	0.76-1.07	0.68-0.97	1.28-1.68	0.63-0.92
	Barbados	1.31-1.79	2.23-2.84	1.47-1.97	1.02-1.45	1.23-1.70	0.69-0.77	1.22-1.68	0.94-1.35	1.26-1.73
	Puerto Rico	2.08-2.63	2.21-2.77	0.79-1.13	1.58-2.06	1.42-1.87	0.46-0.73	0.96-1.07	0.76-1.10	1.31-1.75
	Bahamas	3.94-4.58	4.76-5.46	0.70-0.99	0.56-0.81	0.98-1.31	0.93-1.26	1.08-1.43	1.07-1.15	1.46-1.86
	Bermuda	3.11-3.72	3.23-3.85	0.49-0.75	1.14-1.52	1.36-1.77	0.87-1.21	1.22-1.62	1.80-2.27	0.78-0.84

Spatial Analyses

Mantel tests identified significant positive correlation between pairwise estimates of F_{st} and the Euclidian distance among localities ($p<0.001$; Figure 4.6A), suggesting that geographic distance serves as an isolating mechanism for populations of *G. ventalina*. Furthermore, modeled oceanographic connectivity was negatively correlated with pairwise F_{st} ($p<0.001$; Figure 4.6B) and positively correlated with long-term migration among regions ($p=0.038$), indicating that ocean currents likely serve as important means of connectivity.

Analyses of spatial autocorrelation also identified significant positive genetic structure, and provide a more detailed view of how this structure changes over increasing geographic distance. A correlogram of the autocorrelation coefficient (*r*) across a range of fixed distance classes (Figure 4.7A) shows significant positive autocorrelation at the shortest distance class (50 km), decreasing steadily over the next three distance classes, and passing through zero at 250 km. This distance, at which *r* first crosses the x-axis, provides an estimate of the extent of positive genetic structure (Sokal and Wartenberg 1983, Smouse and Peakall 1999, Peakall et al. 2003). The dispersal neighborhood, defined as the region throughout which the larval pool is well-mixed, can be estimated from the autocorrelogram as the region over which the autocorrelation coefficient (*r*) remains roughly constant, before it begins to decline (Epperson 2005). The autocorrelogram of *G. ventalina* (Figure 4.7A) shows no such initial plateau, but declines consistently from the first distance class through zero. This pattern held for distance class sizes as low as 1 km (data not shown), the maximum resolution possible with this dataset, suggesting that most *G. ventalina* propagules disperse less than 1 km from their parent colonies. At larger distance classes, the correlogram fluctuates between negative and positive values. Though the specific trajectory of the correlogram is typically not interpreted beyond the first x-

intercept, this fluctuation is also consistent with positive spatial structure at shorter distance classes (Smouse and Peakall 1999).

A plot of the autocorrelation coefficient across a range of increasing distance class sizes is shown in Figure 4.7B. This approach, which is less sensitive to distance class size, provides an alternative estimate of the extent of positive spatial genetic structure (Peakall et al. 2003). Autocorrelation is, again, highest for the smallest distance class and decreases steadily to near-zero at a distance class size of 900 km. Thereafter, *r* decreases much more slowly and does not become insignificant until a distance class size of 2500 km. Based on these two alternative analyses of spatial autocorrelation, the limit of positive spatial genetic structure for *G. ventalina* is estimated to occur between 250 and 900 km.

DISCUSSION

The multiple analyses of population structure presented here consistently illustrate that, although dispersal by *Gorgonia ventalina* results in significant gene flow across thousands of kilometers, populations are not panmictic and can be differentiated across distances less than one kilometer. Throughout the range, genetic differentiation among localities was positively correlated with geographic distance and negatively correlated with dispersal probability (Figure 4.6). These trends suggest that distance is an important isolating mechanism for *G. ventalina*, while dispersal via ocean currents is a likely means of connectivity among populations.

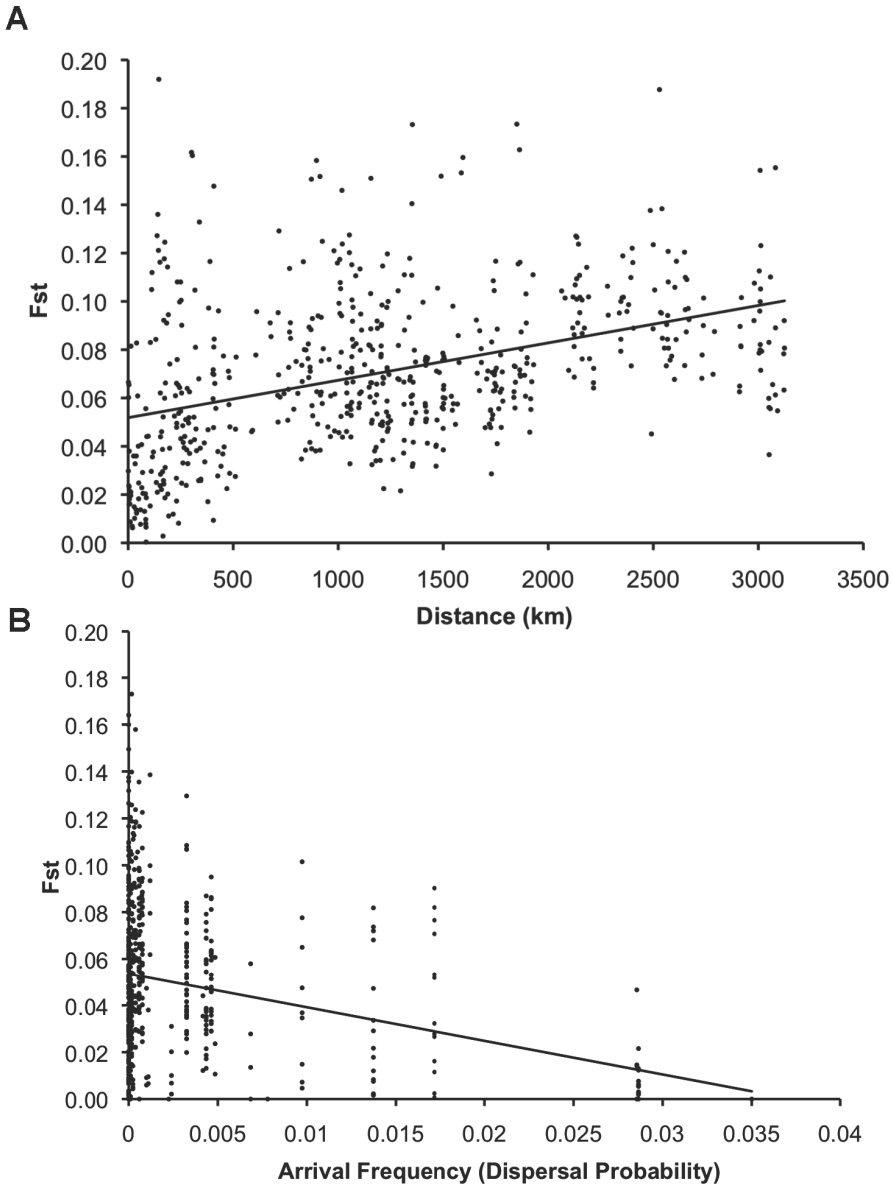

Figure 4.6: Correlation of genetic differentiation (F_{st}) with geographic distance and dispersal probability for *G. ventalina*. **A.** Linear regression of pairwise Fst between each of the 35 sampling localities versus Euclidean distance ($R^2 = 0.17$, Mantel test $p<0.001$). **B.** Linear regression of pairwise Fst and simulated dispersal probability ($R^2 = 0.07$, Mantel test $p<0.001$)

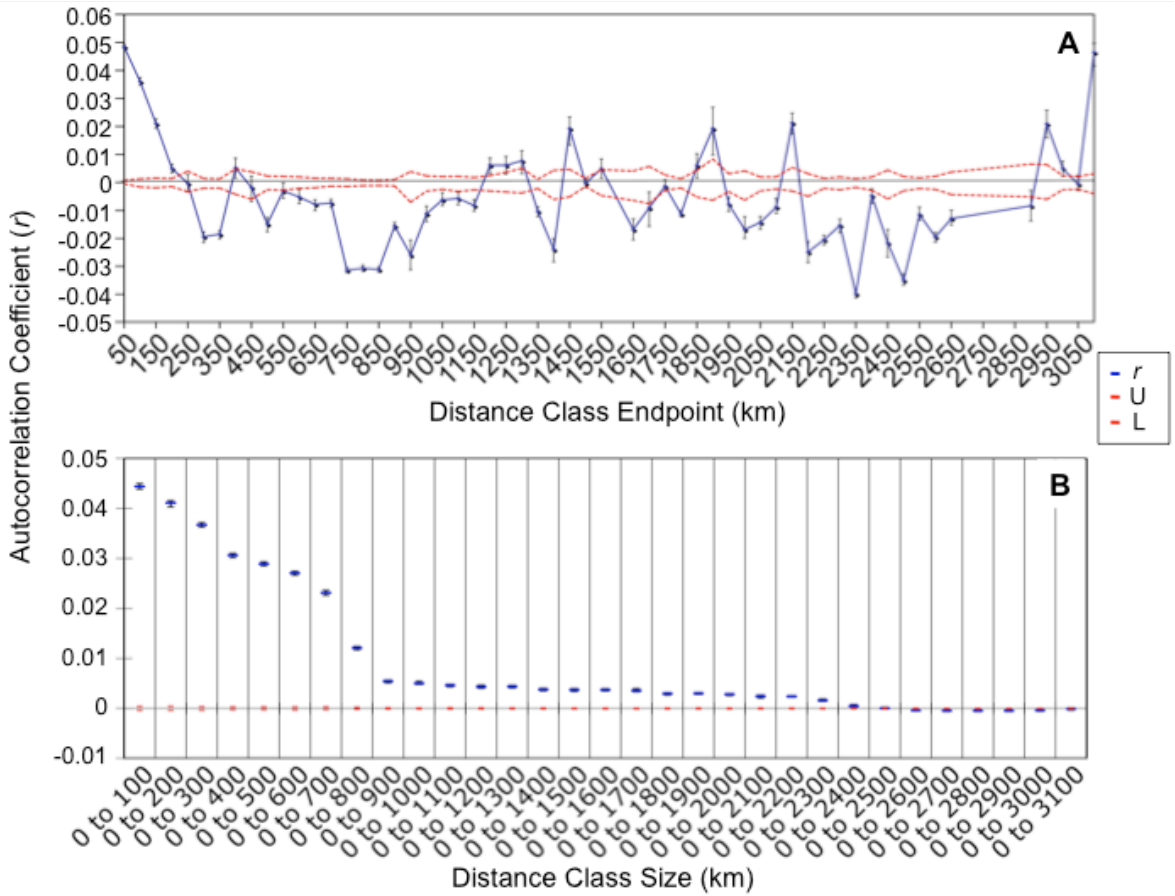

Figure 4.7: Spatial autocorrelation of genetic distance among *Gorgonia ventalina*. **A**. Correlogram of the autocorrelation coefficient (*r*) over increasing distance classes of fixed size (50 km). **B.** Autocorrelation coefficient (*r*) over multiple distance classes of increasing size. Error bars about *r* (in blue) depict 95% confidence intervals based on 1000 bootstrap trials. U and L (in red - nearly overlapping so appear as only one mark) delimit the upper and lower bounds respectively of the 95% confidence interval about the null hypothesis of no spatial autocorrelation based on 1000 random permutations of the data.

Differentiation and Biogeography of G. ventalina *Populations*

The dominant ocean currents throughout the Caribbean and far-western Atlantic are largely continuous and unidirectional (Joyce et al. 2001, Figure 4.4), and the species range of *G. ventalina* can be construed along the trajectory of these currents. Most flow enters the Caribbean between the Windward Islands in the East, moves west/northwest across the Caribbean Basin (Caribbean Current), through the Gulf of Mexico (Loop Current) and Straits of Florida (Florida Current), and joins the Gulf Stream heading north/northeast. Along this route, the contiguous distribution of genetic groups identified by Bayesian clustering loosely delimits five interconnected regional populations of *G. ventalina* (Figure 4.4). At the opposite ends of the range, the Windward Islands (Tobago and Barbados) in the East, and Bermuda, in the North, were highly differentiated from sites in the center (Figure 4.4 - Purple & Yellow Clusters, respectively). In general, localities in the middle of the range showed greater admixture and shared cluster membership, though regional patterns of differentiation were still quite clear. An abrupt juncture separated the Windward Islands from Puerto Rico and Curacao immediately to the west. Puerto Rico shared common cluster membership with the Bahamas to the northeast (Figure 4.4 – Blue Cluster) indicating substantial gene flow and admixture throughout the northern edge of the Caribbean Basin. Interestingly, there was also substantial gene flow apparent between the Bahamas and Florida Keys (Figure 4.4 - Red & Blue Clusters), although the latter were united by the occurrence of a single cluster uncommon throughout the rest of the range (Figure 4.4 – Orange Cluster). The apparent connectivity between the Florida Keys and Bahamas is notable, given that these regions are separated by the strong Florida Current, which could inhibit the dispersal of propagules between them. In the western Caribbean, localities from Panama and the Mesoamerican Barrier Reef were dominated by two clusters (Figure 4.4 - Brown & Green Clusters) that rarely occurred

elsewhere. The apparent connectivity across this broad region is consistent with local retention and mixing of propagules via the seasonally variable eddies and gyres that occur in the Southwestern Caribbean (Andrade and Barton 2000, Richardson 2005).

Estimated Dispersal of G. ventalina

Based on analyses of spatial autocorrelation (Figure 4.7A and 4.7B), the extent of positive genetic structure for *G. ventalina* is estimated to occur between 250 and 900 km, indicating that gene flow via dispersal does not commonly exceed this range. However, this range represents an upper limit, and most dispersal likely occurs over much shorter distances. Though the sampling scheme of this study is insufficient to resolve the precise neighborhood of dispersal, our results suggest that most *G. ventalina* propagules disperse less than 1 km from their parent colonies.

Estimates of long-term migration illustrated significant connectivity among all regions, and 75% of pairwise estimates among regions were asymmetric (Table 4.4). In some cases the directionality of net migration was consistent with expectations based on patterns of prevailing currents. For example, the Mesoamerican Barrier Reef and Florida Keys were identified as sinks for migration from all localities "upstream". However, there were also several counterintuitive patterns. Namely, Tobago and Barbados were not consistently identified as source populations, and Bermuda was not consistently identified as a sink. These patterns could reflect a genuine complex history of inter-regional connectivity. Alternatively, they could be spurious artifacts of an inadequate model of microsatellite mutation. Coalescent methods of estimating migration are based on assumptions regarding the mutational mechanisms of the genetic markers they employ. For our data, we used a stepwise mutational model, as microsatellites are thought to evolve via the incremental gain or loss of repeats. However, the evolution of microsatellites is complex and can be prone to homoplasy

(Ellegren 2004). Patterns of long-term connectivity among *G. ventalina* populations could be more confidently assessed using more conservative sequence-based markers with better-understood mutational mechanisms. In contrast to long-term migration rates, estimates of recent migration (i.e. over the past two generations) show that regional populations are almost exclusively self-recruiting, with the exception of Panama, which received a significant fraction of migrants from the Mesoamerican Barrier Reef to the North. This result, combined with the significant differentiation of most localities and small dispersal neighborhood, indicates that, although regional populations are evolutionarily coupled via gene flow, they are likely to be demographically and, hence, ecologically independent.

Genetic Diversity of G. ventalina *Populations*

Gene diversity (expected heterozygosity) and allelic richness of *G. ventalina* varied by as much as 17% throughout the range (Table 4.1, H_e=0.58-0.69) and were positively correlated with latitude (Figure 4.2). One striking feature of this trend is the relatively high diversity of Bermuda. The combination of isolation, extreme environmental conditions, and genetic drift associated with low population densities are expected to result in reduced genetic diversity at the edges of species ranges (Hoffmann and Blows 1994). However, substantial immigration can maintain high diversity in peripheral populations, and fluctuating environmental conditions at range boundaries may impose balancing selection, thereby increasing diversity relative to population centers (Brussard 1984, Lesica and Allendorf 1995). Both of these are plausible explanations for the high relative diversity of Bermuda. Based on the dominant ocean current patterns discussed above, Bermuda is the farthest "downstream" site in the range, and may therefore act as a sink for genetic diversity. Moreover, sea temperature variability is much greater in Bermuda than the Caribbean

Basin (Sheppard and Rioja-Nieto 2005) and routinely approaches the lower tolerance limit of tropical corals (~18°C). In agreement with a source/sink dynamic driven by prevailing current patterns, Tobago and Barbados, the farthest "upstream" localities at the eastern limit of the species range, had among the lowest gene diversity and allelic richness observed (Figure 4.2).

Overall genotypic diversity was high and there were no duplicate multilocus genotypes observed, indicating that sexual outcrossing is the predominant, if not exclusive, mode of reproduction for *G. ventalina*. This stands in contrast to numerous other coral species for which clonal reproduction and/or self-fertilization are common (Highsmith 1982, Heyward and Babcock 1986, Fautin 2002). Most reported asexual reproduction in corals occurs via disturbance-mediated fragmentation of brittle adult colonies. Given that *G. ventalina* colonies are flexible and not easily broken, the absence of clones is perhaps not surprising. Moreover, as *G. ventalina* is dioecious (Petes et al. 2003, Fitzsimmons-Sosa et al. 2004), self-fertilization is not possible. However, asexual reproduction can also occur via budding, fission, and the release of clonal planulae, and our results indicate that none of these mechanisms are likely for *G. ventalina*. The high genotypic diversity resulting from sexual recombination may provide greater adaptive potential and resilience in the face of selective pressures such as disease. However, sexual species will be more susceptible than clonal species to density dependant phenomena such as the Allee effect (Allee 1949) and may recover from disturbance more slowly.

Comparative Population Structure of G. ventalina *and* Symbiodinium *B1/184*

The population genetic structure of *Symbiodinium* B1/184 associated with *G. ventalina* has been previously described based on the same tissue samples used in this study (Chapter 3), and there are several features common to both symbiotic partners.

Both exhibit a positive correlation between geographic and genetic distance (Figure 3.6 and Figure 4.6), and the maximum extent of spatial structure is similar for both (~200-900 km, Figure 3.7 and Figure 4.7). Pairwise estimates of Fst among localities are strongly correlated for *G. ventalina* and *Symbiodinium* (Figure 4.8; Mantel test: r=0.55, p=0.001), and some patterns of inter-regional connectivity are common to host and symbiont (Figure 3.3 and Figure 4.4). For example, both *G. ventalina* and *Symbiodinium* showed little connectivity between the Mesoamerican Barrier Reef and the Florida Keys, suggesting that the Yucatan Channel may represent an important divide between the Caribbean Basin and localities further downstream. Additionally, populations of both symbiotic partners are strongly differentiated between the Windward Islands and Puerto Rico. This break is noteworthy, as previous studies of fish (Colin 2003, Taylor and Hellberg 2003a, 2006) and corals (Baums et al. 2005, Baums et al. 2006, Galindo et al. 2006) have identified a similar east/west juncture near Puerto Rico, suggesting that this may be a common biogeographic divide for many Caribbean organisms. The exact placement of this break, however, has not been consistently resolved, with some studies placing Puerto Rico to the east of the divide and others to the west. Our results resolutely indicate that, for *G. ventalina* and its algal symbiont, this biogeographic break falls somewhere east of Puerto Rico.

Despite these similarities, however, the population structures of *G. ventalina* and its algal symbiont are not completely congruent. Most significantly, the degree of population differentiation is dramatically higher for *Symbiodinium* than for *G. ventalina*. This disparity is most apparent in estimates of F_{st} and R_{st}, which are, on average, more than three times higher for *Symbiodinium* than *G. ventalina* (Table 3.3 and Table 4.3). In addition, there are key connectivity patterns that are distinct for host and symbiont. For example, populations of *G. ventalina* in the Bahamas and

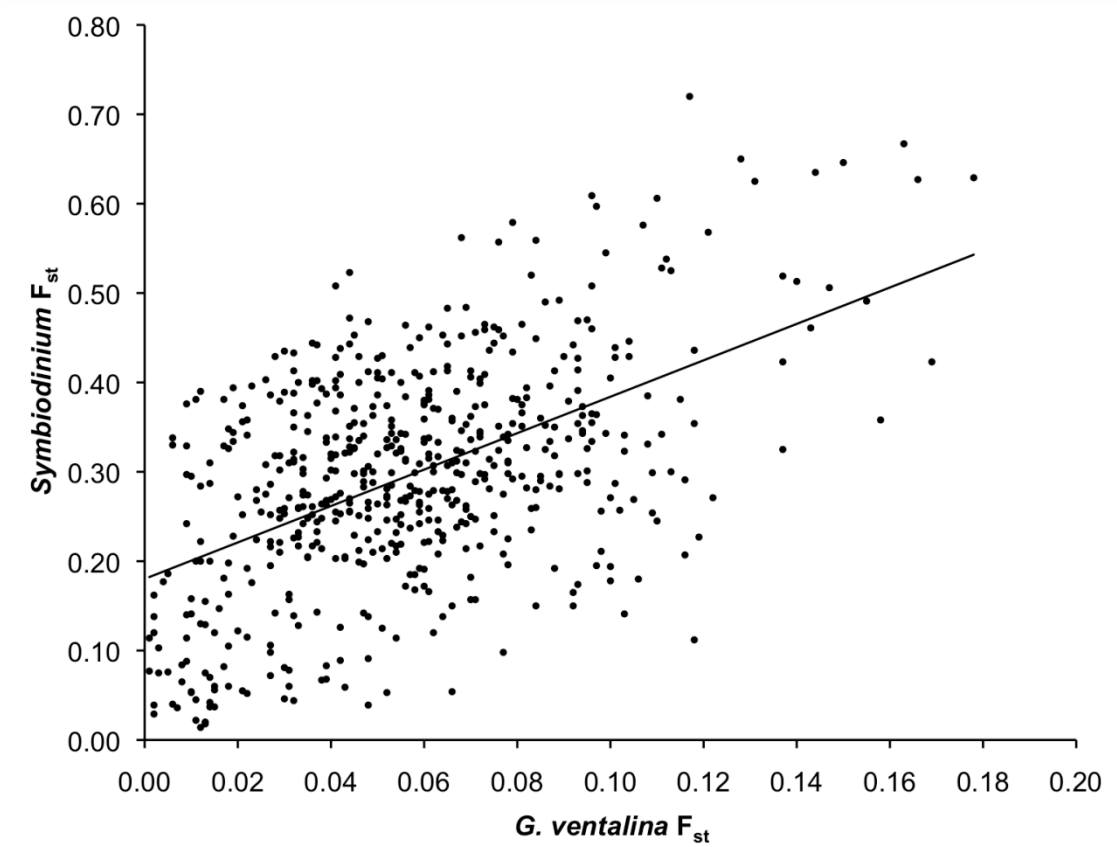

Figure 4.8: Correlation of pairwise F_{st} estimates for *G. ventalina* and *Symbiodinium*. Estimates are based on the same samples from the same localities across the Caribbean and Western Atlantic, and illustrate a significant positive correlation (Mantel test: r=0.55, p=0.001)

the Florida Keys appear to be connected by substantial gene flow, whereas *Symbiodinium* populations from these two regions are among the most highly differentiated in the range. In contrast, *Symbiodinum* is connected across regions that divide populations of the host. One occurs in the southeastern Caribbean between the Windward Islands and Curaco. A second occurs in the North, between Florida and the Bermuda, although some evidence suggests that this apparent connectivity may be due to founder effects among *Symbiodinium* populations in the Bermuda, and not substantial ongoing gene flow (see Chapter 3). Finally, in contrast to the positive correlation between gene diversity and latitude observed for *G. ventalina*, *Symbiodinium* showed the opposite trend (Figure 3.2 and Figure 4.2). The positive correlation observed for *G. ventalina* is concordant with a rangewide source/sink dynamic driven by ocean currents, whereas the negative latitudinal trend in *Symbiodinium* is more consistent with a tropical center of distribution with reduced diversity near latitudinal range limits. The contravening geographic trends of host and symbiont diversity may have complex consequences for the adaptive capacity of the intact symbiosis. Assuming individual variants of host and symbiont can freely combine, the genetic diversity of each partner will have a multiplicative effect on the diversity of the holobiont, which would be predicted to be highest in the middle of the range of *G. ventalina*.

Taken together, the similarities between the population structure of *G. ventalina* and the *Symbiodinium* it harbors suggest that ocean currents are a likely means of connectivity for both partners. On the other hand, the disparities in population structure provide strong evidence that the dispersal mechanisms of host and symbiont are not coupled and that symbiont transmission occurs horizontally. These observations are consistent with a number of other aspects of the biology of these

organisms. Histological evidence suggests that *G. ventalina* is a dioecious broadcast spawner (Petes et al. 2003, Fitzsimmons-Sosa et al. 2004), a reproductive mode that is correlated with aposymbiotic larvae and a long pelagic larval duration relative to species of brooding coral (Knowlton and Rohwer 2003). *Symbiodinium* are capable of active motility (Freudenthal 1962) and have been detected in the water column (Coffroth et al. 2006, Littman et al. 2008, Manning and Gates 2008, Porto et al. 2008), but are much more common in sediments immediately surrounding reefs (Littman et al. 2008). These characteristics would provide opportunities for both symbiotic partners to disperse via ocean currents, but the probability would be much greater for the host. In essence, both coral and symbiont may use the same "highways" to get from place to place, but *G. ventalina* spends more time on "the road" than the algae it hosts.

CONCLUSIONS

The results presented here add to the growing evidence that, contrary to previous expectations, many marine populations are not well mixed, but show considerable structure over short distances. The specific population structure of *G. ventalina* and its *Symbiodinium* corroborate previously reported biogeographic breaks in the Carribbean (e.g. an east/west divide near Puerto Rico), and identify new breaks that warrant further study in additional taxa (e.g. a divide near the Yucatan Channel, and the isolation of Bermuda). Population subdivision has both fundamental and applied implications for the biology of a species. To the extent that subpopulations are demographically independent, their ecological and evolutionary dynamics will be uncoupled. Species with subdivided populations have greater capacity to adapt to local conditions, but may be more sensitive to disturbance. The independent population structure of *G. ventalina* and its *Symbiodinium*, highlight the importance of

considering both symbiotic partners when designing conservation and management plans for corals. For example, an adequate management strategy for *G. ventalina* might, at a minimum, include a network of protected areas that covers the regional populations of host and symbiont and is spaced closely enough to accommodate the small estimated dispersal neighborhoods. Such considerations will become increasingly important as spatially explicit management plans are more broadly implemented for the protection of threatened marine taxa.

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CHAPTER 5:

Fine-scale diversity and dynamics of intracolony *Symbiodinium* populations hosted by the Caribbean sea fan coral, *Gorgonia ventalina*

ABSTRACT

Many tropical corals exist in obligate symbiosis with dinoflagellate algae of the genus *Symbiodinium*. Significant genetic and ecological diversity exisits within *Symbiodinium*, and it has been hypothesized that corals may "adapt" to rapid environmental change by switching the type of symbiont they host. The majority of coral species studied to date have been found to not change their symbionts, however, most previous studies were based on genetic markers that resolve only broad *Symbiodinium* lineages (clades), allowing the possibility that symbiont switching may occur at finer scales of diversity. Here we use hypervariable microsatellite markers to survey *Symbiondinium* populations within individual colonies of the Caribbean sea fan coral, *Gorgonia ventalina*. This study is the first to investigate the intracolony dynamics of *Symbiodinium* at the level of individual clones. We show that substantial fine-scale *Symbiodinium* diversity occurs among and within individual colonies of *G. ventalina*, and that symbiont types can switch in response to light stress, heat stress, and reciprocal transplant across depth. The functional significance of such fine-scale changes for the intact symbiosis remains unclear, although our data suggest that stress-related mortality may be lower among corals that change their symbionts than those that do not. Although some *G. ventalina* colonies changed their symbiont in response to environmental stress, most did not, suggesting that, even if fine-scale symbiont switching confers adaptive benefits on the coral, the ecological significance of this phenomenon may be limited.

INTRODUCTION

Microbial symbioses are vital for the development and health of most metazoans. By combining novel metabolic capabilities of two disparate organisms, symbiotic associations effectively represent a new unit of biodiversity (the holobiont) with greater phenotypic complexity and broader niche potential than either partner possesses alone (Trench 1979, Cavanaugh 1994, Moran 2007). Accordingly, the specific identity of symbiotic partners can have important implications for the functional characteristics of the symbiosis, and the possible permutations of different host-symbiont combinations can augment the adaptive capacity of the holobiont.

Shallow water tropical corals provide an excellent and important example of the expanded complexity and diversity that microbial symbioses can create. Many corals exist in obligate, mutualistic endosymbiosis with dinoflagelate algae of the genus *Symbiodinium* (Trench 1979). *Symbiodinium* reside within the coral's gastrodermal cells, where they translocate as much as 95% of their photosynthetic product to the host in the form of glycerol, carbohydrates, and peptides (Muscatine 1967, Muscatine et al. 1984). Additionally, the active uptake of carbon dioxide by *Symbiodinium* helps to maintain the alkaline environment necessary for the deposition of calcium carbonate skeletal elements by the host (Pearse and Muscatine 1971). In return, *Symbiodinium* benefit from the consistent source of ammonium and phosphate present in the coral's metabolic byproducts, as well as an enhanced environment for collecting light for photosynthesis (Enriquez *et al.* 2005). This mutualism has allowed reef ecosystems to thrive and diversify in the oligotrophic waters of tropical oceans (Muscatine and Porter 1977),

Due to a paucity of obvious distinguishing morphological characters, *Symbiodinium* was once thought to be a cosmopolitan, monotypic genus (Taylor 1974). However, molecular genetic data have since revealed high diversity and deep

divergence commensurate with that found among distinct orders of free-living dinoflagellates (Rowan and Powers 1992). Phylogenies based on a variety of ribosomal, mitochondrial and chloroplast sequences have congruently resolved eight broad clades, A-H (reviewed in Baker 2003, Coffroth and Santos 2005), each of which contains a diversity of more closely-related phylotypes. Many of these phylotypes exhibit distinct geographic distributions (Baker 2003, LaJeunesse et al. 2004a), host specificity (LaJeunesse *et al.* 2004b), depth zonation (Rowan and Knowlton 1995, Iglesias-Prieto et al. 2004, Frade et al. 2008), and physiology (Iglesias-Prieto and Trench 1997, Robison and Warner 2006, Warner et al. 2006).

Over the past thirty years, tropical corals have suffered serious declines as a result of numerous anthropogenic threats, one of the most serious of which is ocean warming associated with climate change (Hughes et al. 2003, Hoegh-Guldberg et al. 2007). Increased average maximum temperatures of as little as 1°C can precipitate the dissolution of the symbiosis between corals and *Symbiodinium*, a phenomenon known as bleaching (Glynn 1993, Hoegh-Guldberg 1999). Bleaching is often fatal for the coral, and, as episodes of thermal stress are predicted to increase in frequency and severity, the fate of tropical corals will ultimately depend on their ability to adapt or acclimate to rapidly warming oceans. Most reef corals are slow-growing and have long generations times - characteristics that impede adaptation. However, the short generation time and high diversity of *Symbiodinium* may provide a means by which the coral holobiont can rapidly "evolve" to accommodate changing environmental conditions, despite the slow adaptive potential of the host (Rowan *et al.* 1997). One popular and contentious formulation of this notion is known as the "adaptive bleaching hypothesis," which posits that rapid symbiont loss might not always be an unmitigated catastrophe, but may, in some cases, enhance the resilience of the holobiont by providing an opportunity for corals to adopt new *Symbiodinium*, better

suited to new environmental conditions (Buddemeier and Fautin 1993, Ware et al. 1996, Kinzie et al. 2001). Such a shift could hypothetically result from either the acquisition of novel symbiont types from the environment, or, if the coral is host to multiple symbiont types, the redistribution of existing *Symbiodinium* diversity.

Whether via adaptive bleaching or some more gradual shift, a number of studies have documented *Symbiodinium* change in response to environmental stimuli. For example, Baker (2001) found that *Symbiodinium* clades within certain Caribbean coral species were differentially distributed according to depth, and, when these corals were reciprocally transplanted across depth, some colonies bleached and were repopulated with the dominant symbiont clade of their new depth environment. In a separate study, Baker *et al*. (2004) found that corals hosting a relatively unusual clade of *Symbiodinium* (clade D) were more thermally tolerant than colonies of the same species hosting the most common symbiont clade (clade C), and clade D symbionts were much more common on reefs that had recently recovered from severe bleaching episodes. Studies of several species of coral in the Indo-Pacific have shown that thermal tolerance and bleaching sensitivity depend, in part, on the clade of *Symbiodinium* they host (Berkelmans and van Oppen 2006, Ulstrup et al. 2006), and a model of the ecological and evolutionary responses of corals to future climate change predicts that *Symbiodinium* clade diversity may provide corals with sufficient adaptive flexibility to persist throughout the next century of climate change (Baskett *et al.* 2009). These and a majority of other studies on the ecology of *Symbiodinium* have resolved diversity at the level of the "clade." While ecological characteristics appear to segregate at the clade level in select cases, it is clear that clades are relatively coarse phylogenetic categories. To accurately assess the level of *Symbiodinium* variation that is relevant to the adaptive capacity of the coral holobiont, it is necessary to understand how functional diversity is distributed across different hierarchical levels of genetic

diversity. Investigation of finer levels of *Symbiodinium* diversity have shown that phenotypic variation among different phylotypes within a clade may be equal to or greater than variation among phylotypes from different clades (e.g. LaJeunesse 2001, Savage et al. 2002). This has prompted efforts to define *Symbiodinium* "species" (LaJeunesse 2001, LaJeunesse 2005, Correa and Baker 2009, Sampayo et al. 2009) – generally identified as genetically diagnosable exclusive groups with some set of coherent phenotypic or ecological characteristics (e.g. morphology, photophysiology, host specificity, depth range) distinct from other closely related groups. This approach has confirmed that *Symbiodinium* clades are indeed composed of numerous ecologically distinct groups, and at least one study has attributed bleaching susceptibility of the coral holobiont to the "species" of *Symbiodinium* it hosts (Sampayo *et al.* 2008).

Despite numerous reports of intracolony *Symbiodinium* change, however, a recent meta-analysis of studies focusing on the clade or phylotype (~species) level found that most of the 442 coral species examined (77%) hosted only a single phylotype of *Symbiodinium* and did not change symbiont types over time or in response to environmental stress (Goulet 2006). Based on this evidence, it was suggested that coral symbioses are not as flexible as some authors have claimed, and symbiont change is not an adaptive strategy available to most corals. Given that the coral/algal symbiosis is intracellular and highly regulated by the host (e.g. Gates et al. 1995, Jones and Yellowlees 1997, Baghdasarian and Muscatine 2000), it is perhaps not surprising that most corals do not harbor multiple *Symbiodinium* clades or "species", either contemporaneously or sequentially. However, if the symbiotic fidelity observed for most corals is due to specificity on the part of either host or symbiont, it seems likely that this specificity would differentiate among reproductively

isolated units of *Symbiodinium* (i.e. species), but not finer scales of freely-recombining genetic diversity.

Recent surveys of *Symbiodinium* diversity based on hypervariable neutral genetic markers such as microsatellites have revealed high genetic diversity at the population level (Chapter 3, Santos et al. 2004, Magalon et al. 2006, Howells et al. 2009, Kirk et al. 2009, Thornhill et al. 2009). This begs the question of whether such fine-scale *Symbiodinium* diversity might correspond with meaningful ecological diversity, and, if so, whether this diversity can change within a coral colony over time. This study explores the potential functional significance and intracolony dynamics of population-level *Symbiodinium* diversity by surveying the fine-scale genetic identity of *Symbiodinium* within an ecologically important Caribbean coral, *Gorgonia ventalina*, throughout the course of seasonal changes, experimentally applied temperature and irradiance stress, and reciprocal transplant across the upper and lower limits of *G. ventalina*'s depth range. To quantify *Symbiodinium* diversity, we employ seven previously developed microsatellite markers that are highly polymorhphic and capable of resolving *Symbiodinium* diversity well below the phylotype (~species) level (Chapter 2, Andras et al. 2009). Because polymorphism at the population level is continually shuffled by recombination, it is not possible (as it is at higher taxonomic levels) to identify neutral genetic markers that are definitively linked to a particular phenotype. It is posited, however, that, if neutral, population-level *Symbiodinium* diversity is observed to change in response to environmental stimuli, then that neutral diversity is associated with significant functional diversity, which might expand the adaptive potential of the coral holobiont.

METHODS

Study Organism

The common sea fan coral, *Gorgonia ventalina* [Cnidaria, Anthozoa, Octocorallia], is one of the most conspicuous and abundant species of octocoral on Caribbean reefs (Sanchez et al. 1997, Sanchez et al. 1998, Ward et al. 2006), and it is only known to associate with a single phylotype of *Symbiodinium* (phylotype B1/184) throughout its range (Goulet and Coffroth 2004, Kirk et al. 2005, Van Oppen et al. 2005). Highly polymorphic microsatellite markers have recently been developed for *Symbiodinium* B1/184 hosted by *G. ventalina* (Chapter 2, Andras et al. 2009). These markers have revealed high genetic diversity and strong population subdivision across distance and depth (Chapter 3, Kirk et al. 2009), while *Symbiodinium* haplotypes were found to be consistent throughout individual colonies of *G. ventalina* (Chapter 3). Collectively, these characteristics make *G. ventalina* an ideal candidate to investigate longitudinal changes in fine-scale *Symbiodinium* diversity within individual coral colonies.

Seasonal Survey of Intracolony Symbiodinium *Diversity*

To assess the seasonal variability of intracolony *Symbiodinium* populations, 75 *G. ventalina* colonies from an unnamed patch reef in the lower Florida Keys (24°32.84'N x 81°27.40'W, 7 m depth) were marked and sampled throughout a year, at the approximate times of annual maximum and minimum sea temperature (Figure 5.1). In October of 2007 (t=0), shortly after the annual maximum sea temperature, 60 *G. ventalina* colonies in each of five ascending size classes (5-10 cm height, n=20; 11-20 cm, n=10; 21-30 cm, n=10; 31-50 cm, n=10; 51-60cm, n=10) were marked with numbered aluminum tags, and small tissue explants (~2cm x 2cm) were collected from the apical colony edge. This sampling method is known to not cause colony mortality,

and most corals regrow the excised tissue rapidly. The marked colonies were resampled four months later (t=4 months, February 2008), approximately coincident with the annual low mean sea temperature, and again 1 year after the initial sampling (t=12 months, October 2008). Upon collection, tissue samples were immediately frozen and shipped to Cornell University for *Symbiodinium* cell counts and genotyping.

Reciprocal Transplant Experiment

To confirm that *Symbiodinium* populations hosted by *G. ventalina* are differentiated by depth, and to determine whether intracolony *Symbiodinium* populations might shift in response to a dramatic change in depth, 40 colonies of *G. ventalina* were reciprocally transplanted across a depth difference of approximately 15 meters, which is roughly the span of the species depth range. In June 2008, 24 small *G. ventalina* colonies (<20cm height) were collected from a shallow fore reef in the lower Florida Keys (2.5 m depth, 24°6.34'N x 81°25.75'W), and 24 small colonies were collected from a deep reef seven kilometers away (18 m depth, 24°32.450'N x 81°24.92'W). Immediately after collection, each colony was secured via its basal holdfast to an acrylic/PVC rack using two-part marine epoxy, eight colonies to a rack. The racks were bolted to cinder blocks for ballast (Figure 5.2), and deployed to the shallow and deep collections sites. Upon deployment, twenty colonies collected at the shallow site were transplanted to the deep site, and twenty colonies from the deep site were transplanted to the shallow site. Four colonies from each site were returned to their original collection locality to serve as controls for experimental manipulation. A small tissue explant (~2cm x 2cm) was cut from the apical colony edge of each of the 48 colonies immediately after deployment (t=0), six months later (t=6 months), and 12 months later (t=12 months), although one rack at the shallow site containing seven

transplant colonies and one control colony could not be located at the six-month sampling. Tissue explants were frozen immediately after collection and shipped to Cornell University for *Symbiodinium* cell counts and genotyping.

Heat/Light Stress Experiment

To investigate whether the composition of intracolony *Symbiodinium* populations can change in response to environmental stress, colonies of *G. ventalina* were surveyed before and after experimental exposure to sublethal heat or light stress. Thirty-four whole small *G. ventalina* colonies (<20cm height) were collected from an unnamed patch reef in the lower Florida Keys (24°34.13'N x 81°22.91'W, 7 m depth) in April, 2008 and shipped live to Cornell University. At Cornell, the colonies were immediately cut into three clonal replicates of approximately equal size and acclimated in a 4000 liter artificial seawater system (Irradiance: 12hr:12hr, 20 μmol quanta $m^{-2}s^{-1}$; Temperature: 26±0.5°C) for six weeks. During this acclimation period, the wounds from division healed completely, and new growth was evident along the colony margins.

At the start of the experiment, the three clonal replicates of each *G. ventalina* colony were split among three treatments: 1) a *control* group maintained at the same conditions as the acclimation period, 2) a *heat stress* treatment that maintained a constant level of irradiance, but gradually increased the temperature 1°C per day over three days to a final temperature of 29°C, and 3) a *light stress treatment* that maintained the same temperature as the control but tripled irradiance over three days to a final level of 60 μmol quanta $m^{-2}s^{-1}$. Each treatment consisted of three 40-liter tanks connected together via a common 200-liter sump, with replicate fragments randomly distributed among tanks. Water was constantly circulated among all tanks within each treatment, and each tank was equipped with two pumps to maintain

turbulent flow around the corals. Corals were maintained under experimental treatment for 6 weeks, during which time fragments were cleaned when necessary to prevent overgrowth by macroalagae, and 50% of the water in each treatment was changed once per week. To quantify photosynthetic performance of *Symbiodinium* throughout the experiment, the minimum (F_o) and maximum (F_m) dark-adapted (30 minutes) fluorescence yield was measured using an underwater pulse amplitude modulated fluorometer (diving-PAM), and the quantum yield of photosystem II (F_v/F_m) was calculated as (F_m- F_o)/F_m. Quantum yield is a commonly used metric of photochemical efficiency of *Symbiodinium*, and depressed values of F_v/F_m have been associated with photoinhibition and/or photodamage. Fluorescence measurements were taken at the beginning and end of the experiment to evaluate any change in photosynthetic performance in response to heat or light stress. After 8 weeks of treatment, all fragments were harvested and immediately frozen for *Symbiodinium* cell counts and genotyping.

Symbiodinium *Cell Counts*

To quantify the density of *Symbiodinium* cells within each *G. ventalina* sample, a circular tissue explant 1 cm in diameter (0.79 cm^2) was cut from each fragment ~1 cm from the colony edge. The explant was weighed (wet weight), frozen in liquid Nitrogen, and ground to a fine powder with a mortar and pestle. The resulting powder was suspended in 400 μl artificial seawater and stained with 40 μl of Lugol's solution. All cells contained in a 0.1 μl volume were counted from four separate aliquots using a brightline hemacytometer with improved Neubauer ruling at 40x magnification.

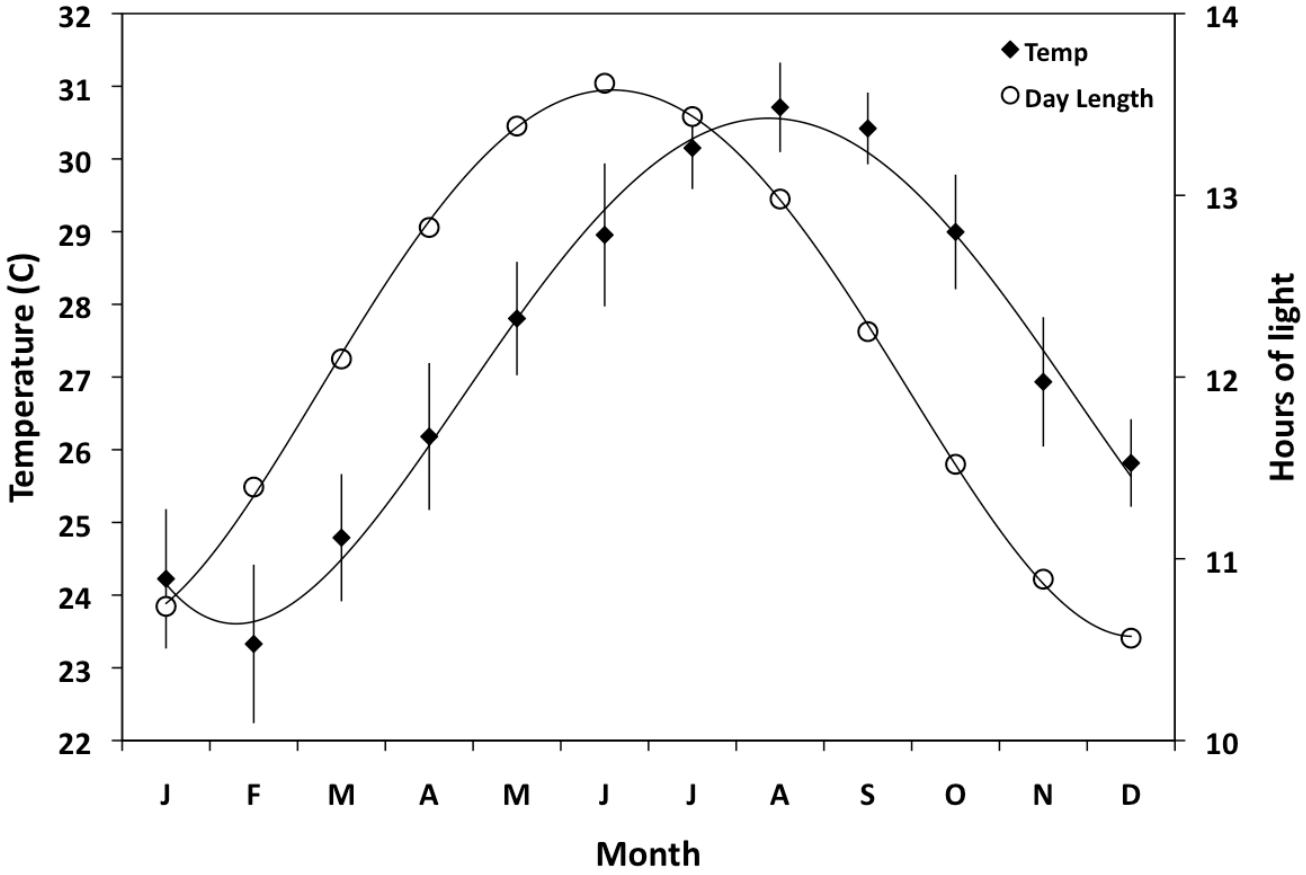

Figure 5.1: Sea surface temperature (◆) and length of daylight (○) in the Florida Keys. Plotted temperatures are monthly averages from 2006-2008 (± S.E.) from the National Data Buoy Center, Station SMKF1, Sombrero Key, Florida. Daylengths were based on sunrise/sunset times obtained from the U.S. Naval Oceanography Command website: (http://aa.usno.navy.mil/data/docs/RS_OneYear.php).

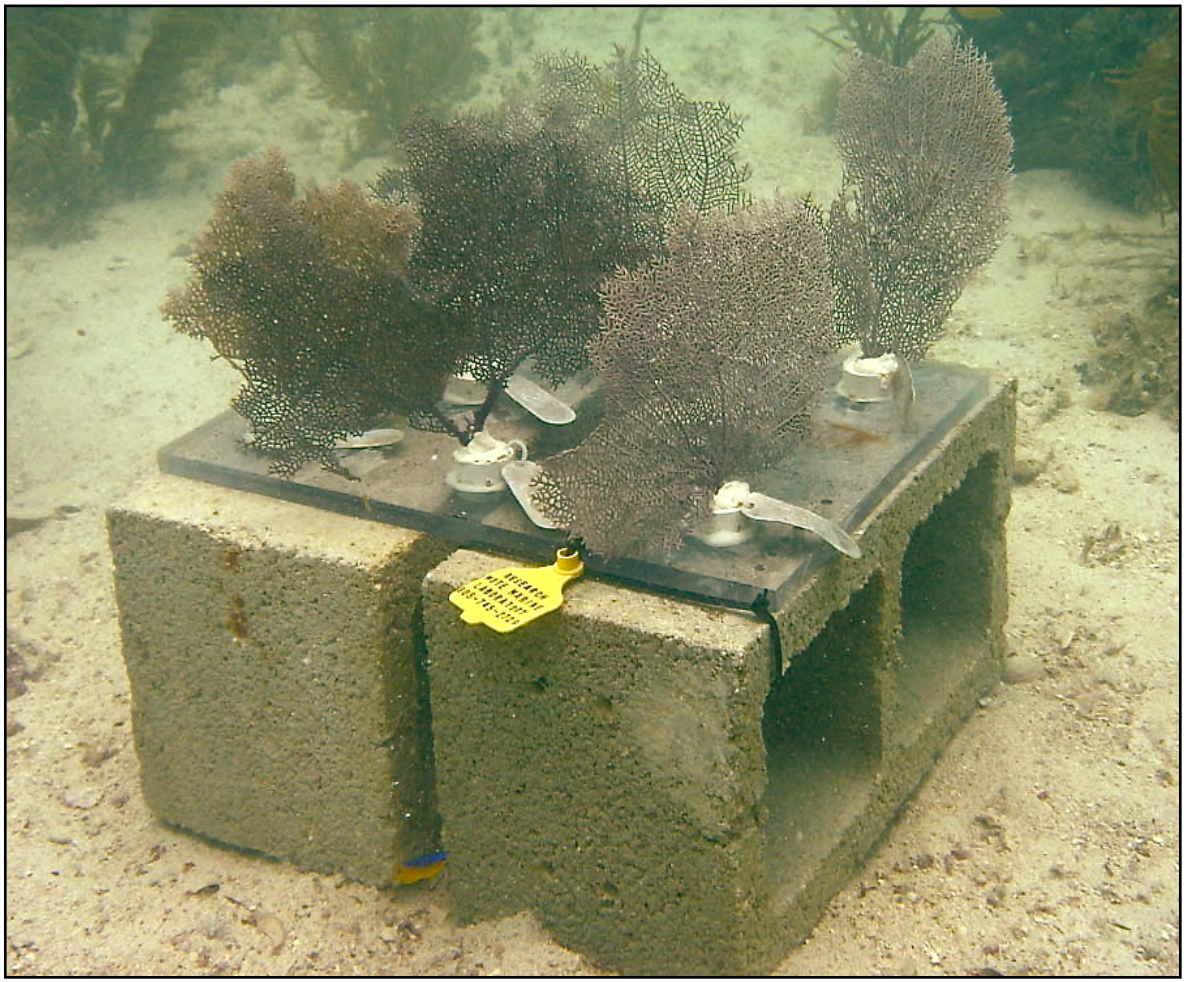

Figure 5.2: Picture of an experimental rack used in the reciprocal transplant experiment. Eight small *G. ventalina* colonies were attached to each rack with marine epoxy. A total of six racks were deployed, three to a 2.5 m site and three to an 18 m site.

Microsatellite Genotyping

Whole genomic DNA was extracted from 1cm^2 tissue subsamples using DNeasy Animal Tissue Kits (Qiagen). Extracted DNA was quantified using a Nanodrop spectrophotometer and diluted to 10 ng/µl with reagent-grade water (Sigma). Each sample was genotyped via polymerase chain reaction (PCR) using primers and run conditions for 10 previously reported microsatellite loci (Chapter 2, Andras et al. 2009). Because genomic extracts from intact coral colonies contain complex mixtures of DNA from both coral and symbiont, all loci were tested extensively on multiple pure cultures of *Symbiodinium* isolated from *G. ventalina* and other cnidarian hosts to ensure they did not cross-amplify (Andras et al. 2009). PCR products were analyzed on an ABI 3100 Automated Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Allele sizes were scored using GENEMAPPER 3.5 (Applied Biosystems) and validated by eye. Two loci exhibited characteristics not well suited to the present study: Locus GV42 occasionally produced inconsistent genotypes within the same sample; and Locus GV44 was monoallelic for all individuals surveyed. These loci were thus excluded from all analyses. All samples that were observed to change *Symbiodinium* haplotype during the course of longitudinal observation were genotyped a second time for confirmation.

Statistical Analyses

As a conservative estimate, we scored colonies as having changed their *Symbiodinium* haplotype only if they showed allele changes at two or more of the eight loci used in this study (at least 28.6%). We used Chi square contingency tests to ascertain the significance of the proportion of colonies exhibiting a change of *Symbiodinium* haplotype relative to controls. Symbiont cell counts and fluorescence

measurements were tested for normality (Shapiro-Wilk) and homoscedascity (Levene), and compared via repeated measures analysis of variance (ANOVA). Significant differences among main effects were analyzed further by Tukey's HSD *post hoc* comparisons. All statistics were performed using the program JMP (version 7.0).

To evaluate genetic differentiation among treatment groups in our experiments, we used a Bayesian clustering approach implemented by the program STRUCTURE 2.2.3 (Pritchard et al. 2000, Falush et al. 2003, 2007). This program probabilistically assigns individuals membership among K populations (where K is specified by the user), without regard to collection locality. We employed a model that allowed admixture, assumed correlated allele frequencies among populations, and did not incorporate population origin information. We conducted 20 independent runs at each K from 1-5. Each run consisted of $2x10^6$ Markov Chain Monte Carlo (MCMC) iterations after a burn-in of $2x10^6$ steps. This run-length was sufficient to achieve stabilization of all relevant summary statistics. To guide our estimation of the number of populations in our dataset, we employed the ΔK method (Evanno *et al.* 2005). This approach identifies the number of clusters (K) at the highest level of population structure by determining the point of maximum concavity (break point) in a plot of posterior probability versus K.

RESULTS

Seasonal Survey

Over the course of the year-long survey, the genetic composition of *Symbiodinium* within most *G. ventalina* colonies was remarkably consistent. Only colonies in the smallest size class (≤10cm) exhibited any change in intracolony *Symbiodinium* haplotype. Over the course of one year, six of the twenty colonies in

the smallest size class (30%) exhibited a change in *Symbiodinium* haplotype at two or more loci (χ^2=13.3, p=0.01), four of which occurred over the first four months (October to June), and two of which occurred over the next eight months. Changes included both losses and gains of alleles, and there were no obvious seasonal patterns or reversions. Bayesian clustering analysis identified differentiation of *Symbiodinium* populations among *G. ventalina* size classes (data not shown), a result that has been previously reported (Chapter 3). However, the majority of colonies within each size class were assigned to a common genetic cluster.

Symbiodinium cell density changed significantly over seasons (Figure 5.3; repeated measures ANOVA: F=346.03, p<0.0001), but there was no effect of *G. ventalina* colony size on cell density (F=1.39, p=0.25). Post-hoc comparisons (Tukey's HSD) identified significantly different *Symbiodinium* densities at each of the three sampling times (Figure 5.3), with the highest abundance occurring in February of 2008 (3.77×10^6 cells/cm^2) followed by October 2008 (2.51×10^6 cells/cm^2), then October 2009 (2.00×10^6 cells/cm^2).

Reciprocal Transplant Experiment

Most transplanted *G. ventalina* colonies appeared healthy throughout the course of the year-long experiment. Four of the 48 colonies died (one shallow control and three deep to shallow transplants), however, three of these colonies were on an experimental block that was partially covered with sediment by storm surge. One colony transplanted from deep to shallow became infected with red band disease (Richardson 1992) after 4 months, though it remained alive throughout the entire experiment. There were no *Symbiodinium* haplotype changes observed in any of the shallow or deep control colonies over the course of the one-year experiment. Haplotype changes were observed in multiple colonies from both transplant groups

(Figure 5.4), though the proportions were not significant. Among corals transplanted from shallow to deep, there were 31 allele changes across 15 individuals during the first six months of transplant, and seven out of 20 (35%) individuals had allele changes at two or more loci. During the second six-month period, there were an additional 20 allele changes among 11 individuals. Over the course of the entire year, 9 out of 20 corals transplanted from shallow to deep (45%) changed at two or more loci (uncorrected: χ^2=2.218, p=0.0682; Yates' correction: χ^2=0.731, p=0.1963). Among corals transplanted from deep to shallow, seven of the 20 colonies (1 experimental block) could not be found at the six-month sampling time, and one colony had died. Of the twelve colonies sampled at 6 months, only two allele changes were detected in one colony (8.3%). However, during the 12-month collection, five of the seven missing colonies were found. Including these samples, there were a total of 32 allele changes among twelve individuals over the course of the year-long transplant, and 7 out of 17 deep to shallow transplants (41.2%) changed at two or more loci over the course of one year (uncorrected: χ^2=2.471, p=0.0580; Yates' correction: χ^2=0.965, p=0.1630).

Bayesian clustering analyses resolved two genetic clusters at each of the three collection times (Figure 5.5). Prior to transplant, *Symbiodinium* from *G. ventalina* colonies were clearly differentiated by depth (Figure 5.5A), with 83% (20/24) of shallow colonies definitively assigned to one cluster (i.e. >75% probability), and 95.8% (23/24) of deep colonies definitively assigned to the other cluster. Cluster assignments remained essentially unchanged after six months (Figure 5.5B) and one year (Figure 5.5C) of transplant, even among colonies whose *Symbiodinium* haplotype had changed at more than two loci.

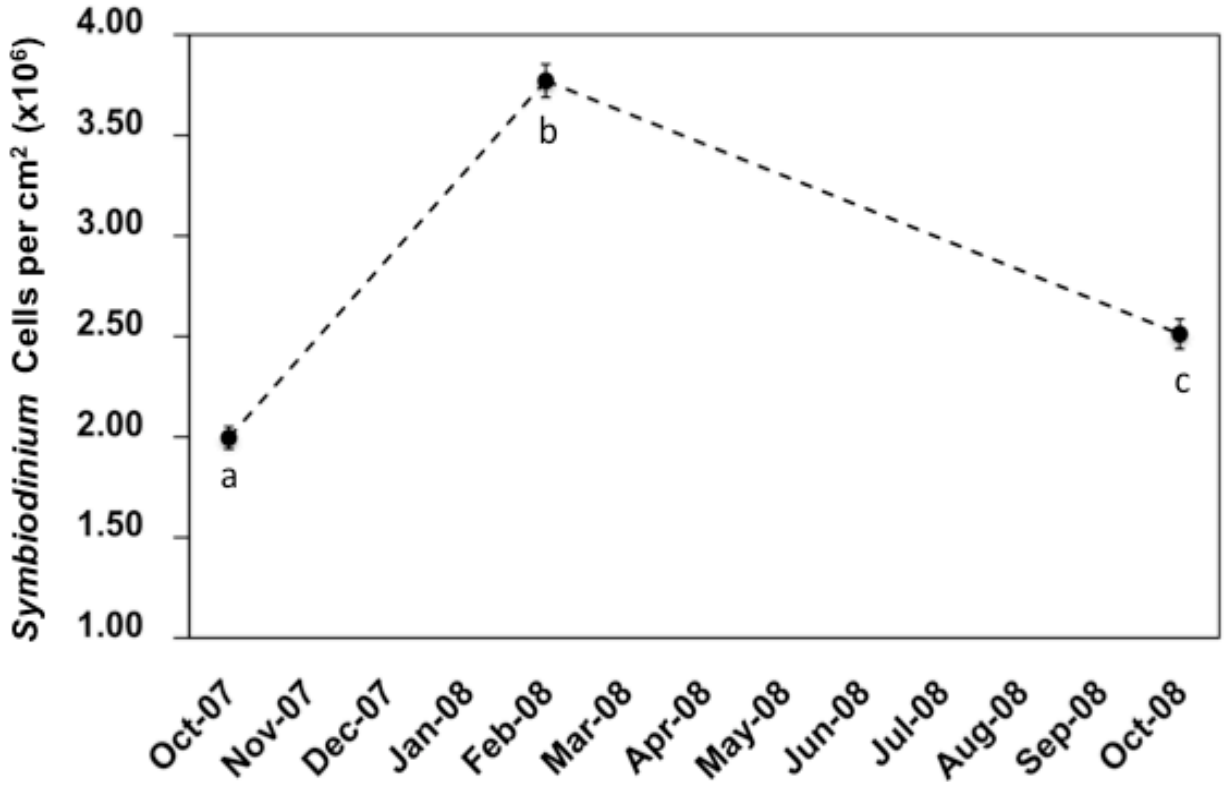

Figure 5.3: Mean seasonal *Symbiodinium* cell densitiesfrom 60 colonies of *G. ventalina* from five different size classes. All size classes were pooled at each of the three sampling times as there were no significant differences among them. There were, however significant differences among sampling times. Points not connected by the same letter are significantly different.

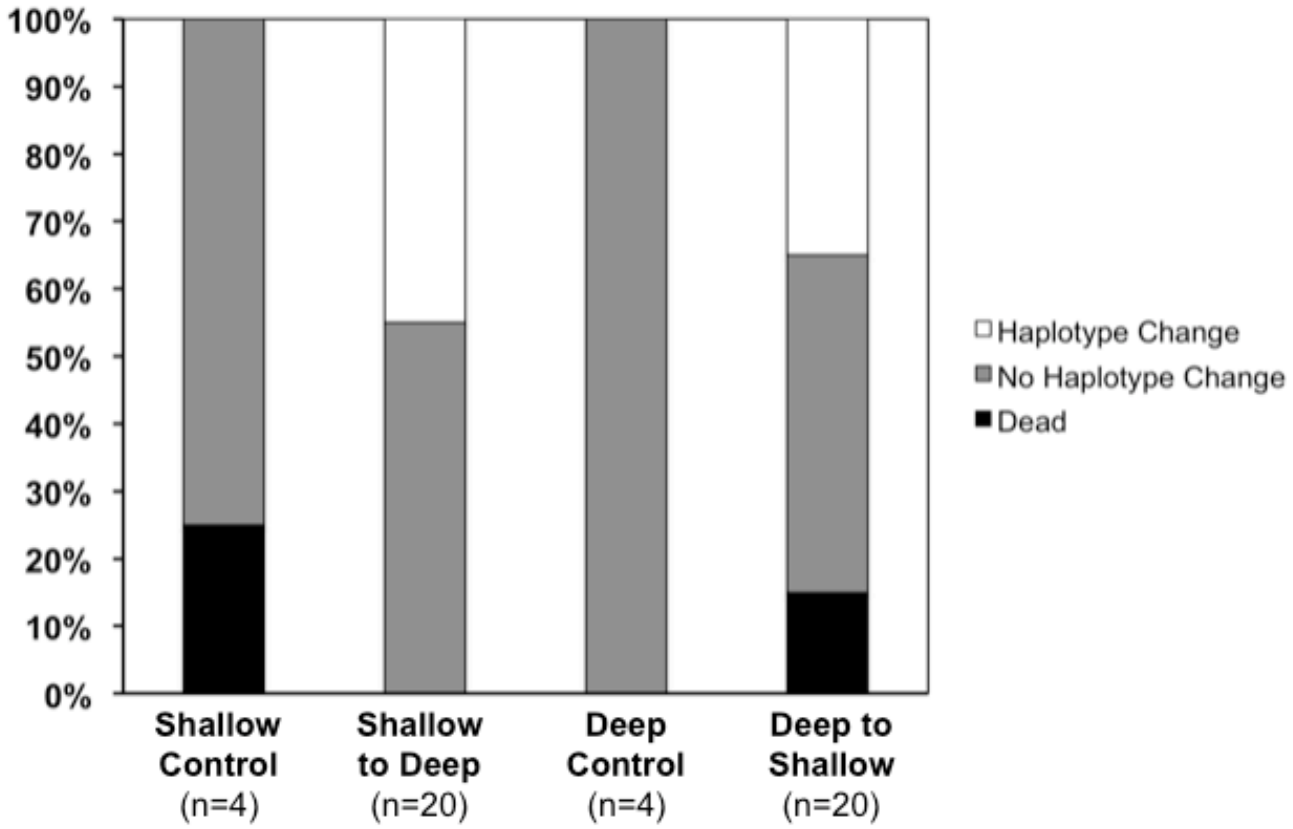

Figure 5.4: Proportion of *G. ventalina* colonies whose *Symbiodinium* haplotype changed over the course of one year of reciprocal transplant across 15m. Changes are not significant relative to controls.

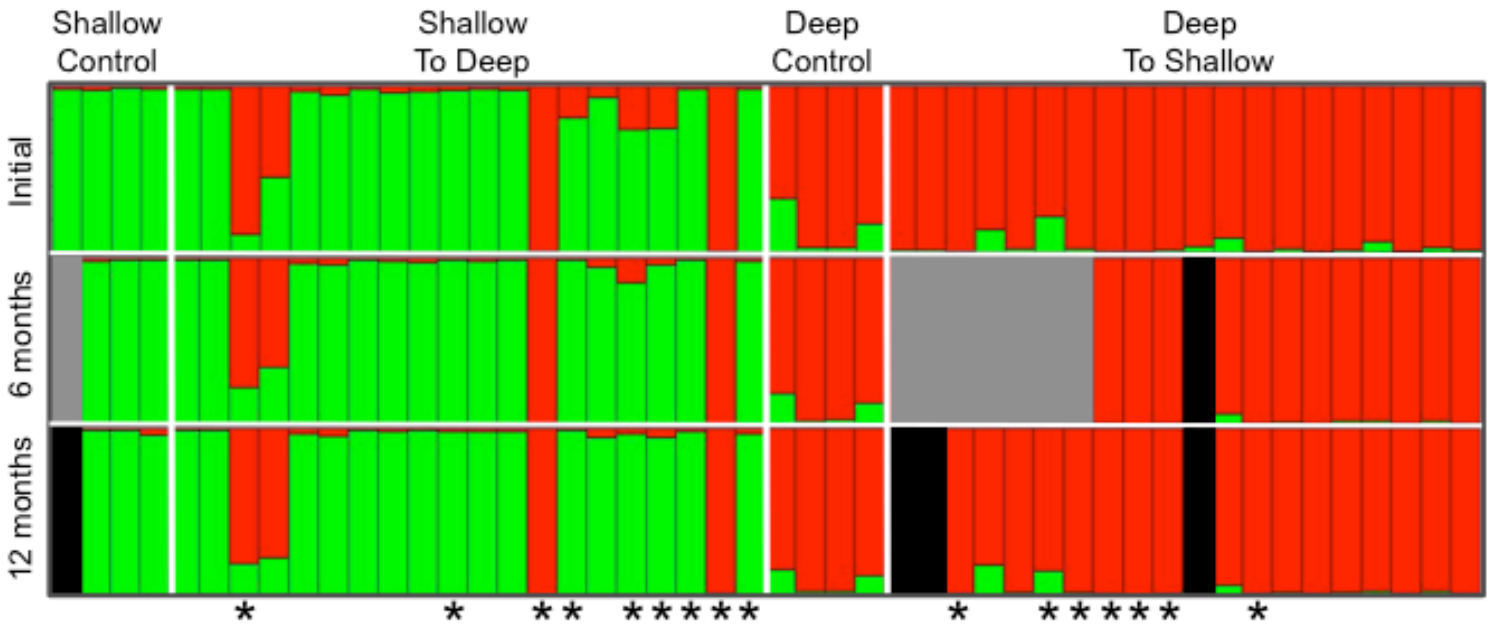

Figure 5.5: Results of a two-cluster Bayesian assignment analysis for *Symbiodinium* over the course of the one-year reciprocal transplant. Each vertical bar represents an individual sample's probabilistic assignment amoung the two color-coded genetic clusters (Red or Green). The three rows of bar plots correspond to the three sampling times (initial, 6 months, 12 months), and individuals are aligned vertically to follow their cluster assignment through time. Bars that are shaded gray denote colonies that could not be located or sampled. Bars that are shaded black denote colonies that died. Asterisks below the charts denote colonies that exhibited changes of *Symbiodinium* haplotype at 2 or more of the 7 microsatellite loci used in this study.

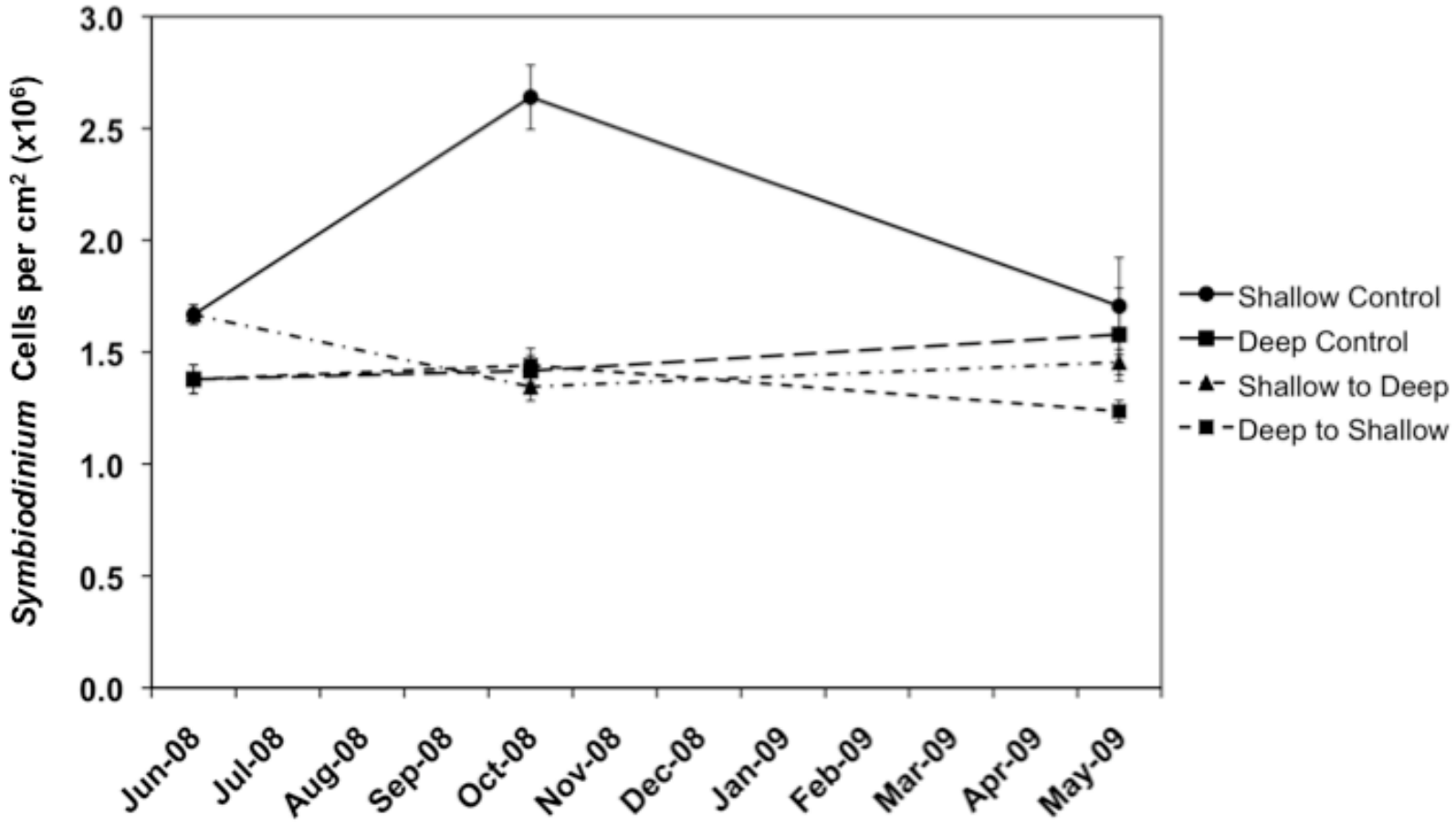

Figure 5.6: Mean *Symbiodinium* cell densities of different treatment groups throughout the one-year reciprocal transplant experiment. There are no significant changes among groups or over time, with the exception of the mean cell density of the Shallow Control group in October, which was significantly higher than all other measurements.

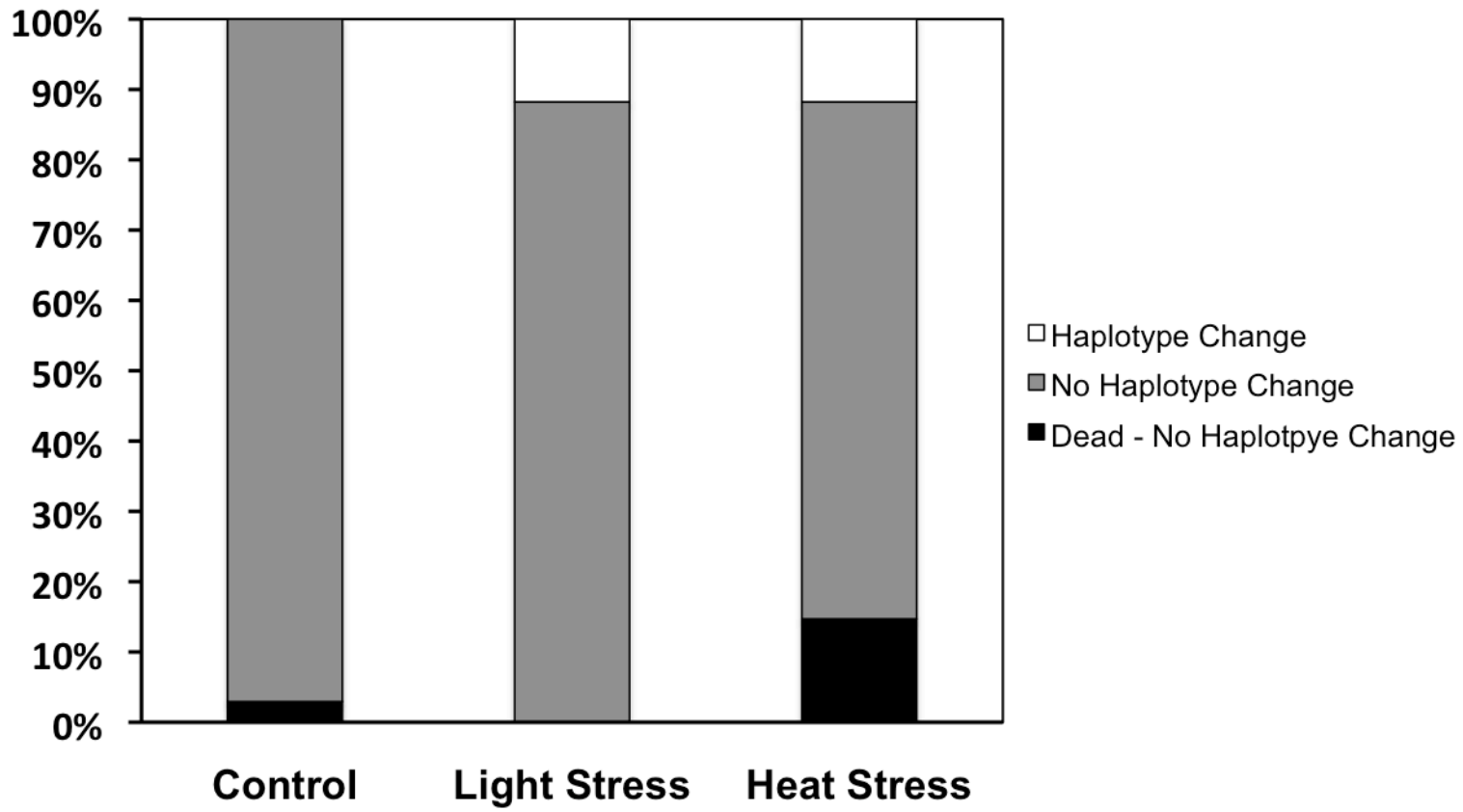

Figure 5.7: Proportion of *G. ventalina* colonies whose *Symbiodinium* haplotype changed for at least two microsatellite loci over the course of six weeks of experimentally applied heat or light stress. Proportions of changes in both experimental groups are significant relative to controls. N=34 for all groups.

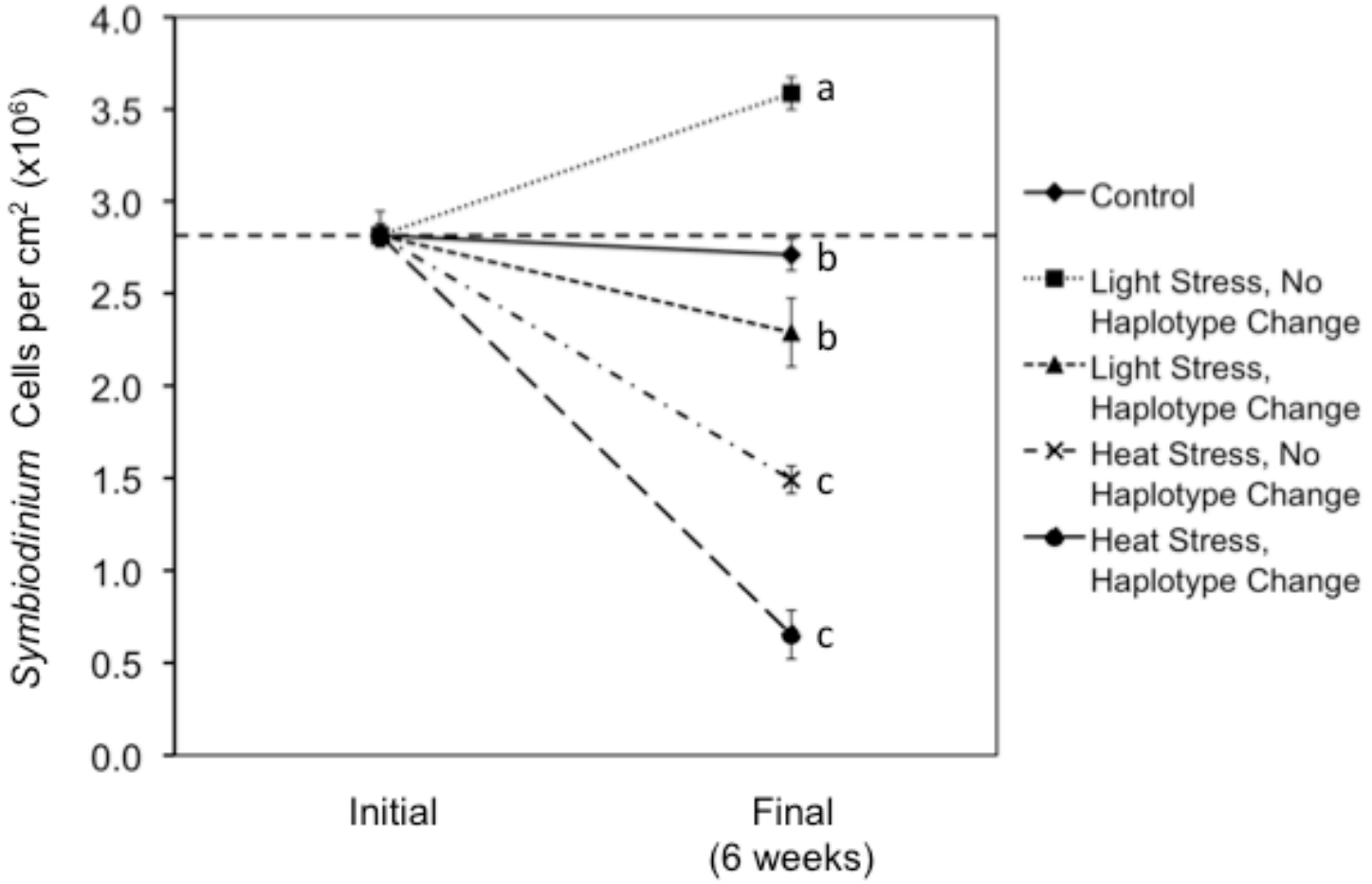

Figure 5.8: Changes in mean *Symbiodinium* cell density of different treatment/response groups over the course of six weeks of experimentally applied heat or light stress. Final counts not connected by the same letter are significantly different.

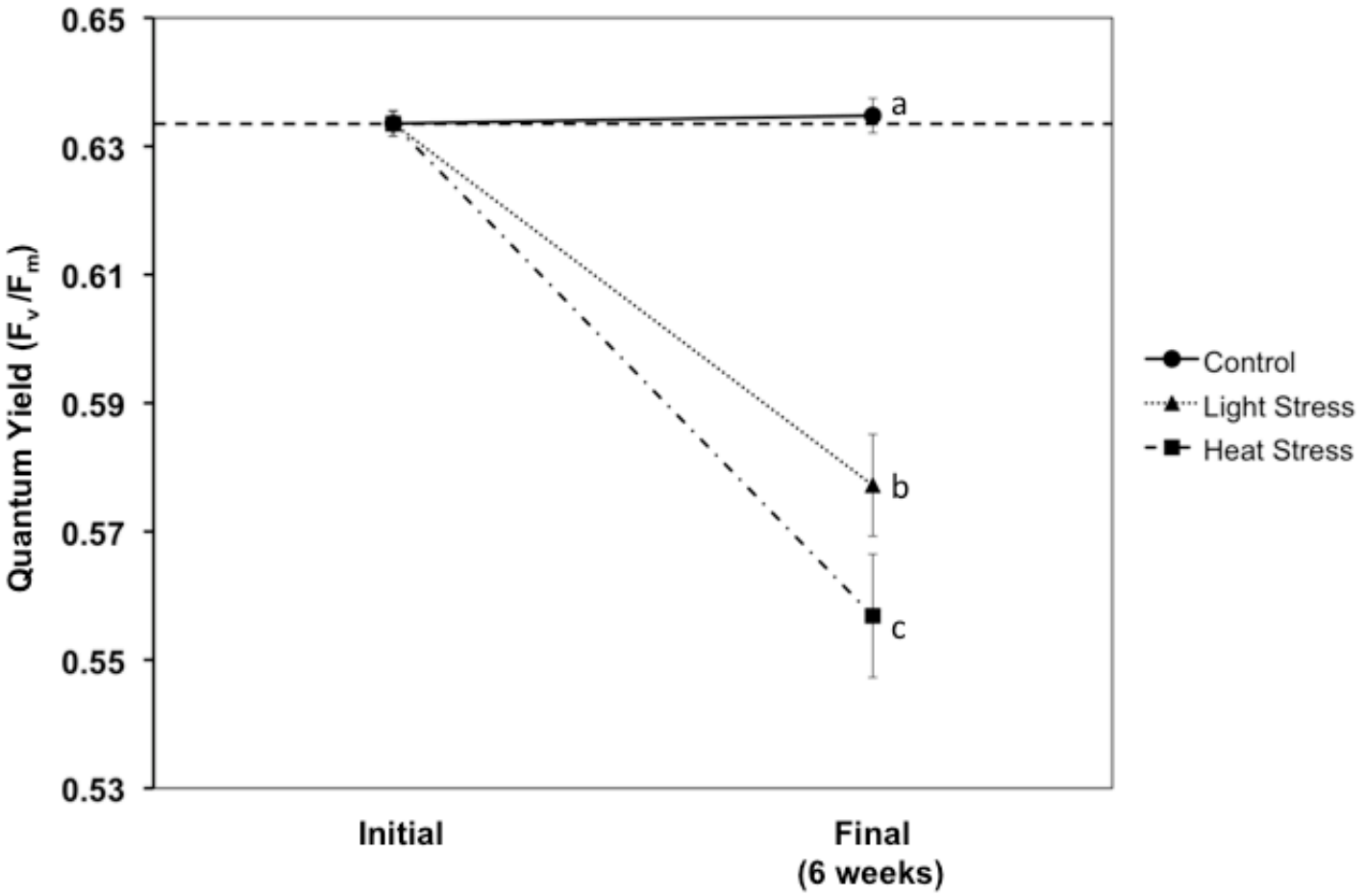

Figure 5.9: Changes in quantum yield of Photosystem II (F_v/F_m) over the course of six weeks of experimentally applied heat or light stress. Final measurements not connected by the same letter are significantly different.

There was an interaction effect of treatment and time on *Symbiodinium* cell densities over the course of the year-long experiment, (Figure 5.6; repeated measures ANOVA, time*treatment: $F=7.98$, $p<0.0001$). The mean cell density of the shallow control group fluctuated significantly throughout the year ($F=171.646$, $p=0.0491$) with higher densities in October relative to June and May. The deep control group showed a similar but much weaker pattern that was not significant. The cell densities of both transplant groups declined consistently over the course of the experiment, but this decline was only significant for those corals transplanted from shallow to deep ($F=11.418$, $p=0.0006$). Within transplant groups, cell densities of colonies that changed haplotype were not significantly different from those that did not change symbionts.

Heat/Light Stress Experiment

Significant numbers of *G. ventalina* colonies changed intracolony *Symbiodinium* haplotype in response to six weeks of heat or light stress (Figure 5.7). Most *G. ventalina* clones appeared to remain healthy and active (polyps extended) throughout the course of the experiment, however, rapid necrosis was observed for one clone from the control group (2.9%) and five clones from the heat stress group (14.7%). These samples were harvested prior to complete mortality, so that *Symbiodinium* within the remaining intact tissue could be genotyped. All clones in the control group maintained consistent *Symbiodinium* haplotypes over the course of the 6-week experiment. Among the group subjected to light stress, a total of 11 clones (32.4%) showed changes in at least one of the eight polymorphic loci surveyed, and 4 (11.8%) showed changes at two or more loci. The group subjected to heat stress showed similar changes with 9 clones showing changes at one or more loci (26.5%), and 4 clones showing changes at two or more loci (11.8%). None of the 5 prematurely

harvested necrotic replicates showed a change in *Symbiodinium* haplotype. Considering only individuals with allele changes at 2 or more of the 8 polymorphic loci, both the heat and light stress treatments significantly altered intracolony *Symbiodinium* composition relative to the control (χ^2=4.25, p=0.039). Two-thirds of the *G. ventalina* colonies that showed any change in *Symbiodinium* haplotype (8/12), showed changes in both heat and light stress groups, suggesting that some host/symbiont combinations may be more likely to change regardless of the stressor. Moreover, the colonies that changed tended to be those apparently hosting multiple strains of *Symbiodinium*. More than seventy percent (12/17) of colonies who initially had multiple alleles per locus changed haplotype in response to heat or light stress. This suggests that the observed changes may be largely due to redistribution of existing fine-scale intracolony *Symbiodinium* diversity and not the acquisition of novel types from the environment. Bayesian clustering analysis resolved only one genetic group for all *Symbiodinium* haplotypes from all treatments, both before and after the experiment, indicating no detectable differentiation among any of the haplotypes observed.

Symbiodinium cell densities behaved differently over the course of the 6-week experiment depending upon treatment (Figure 5.8; repeated measures ANOVA, time: F=50.61, p<0.0001; time*treatment: F=58.56, p<0.0001). Throughout the experiment, *Symbiodinium* cell density did not change significantly in the control group (repeated measures ANOVA, time: F=1.04, p=0.3149). In response to light stress, cell densities increased in colonies whose *Symbiodinium* haplotype remained constant throughout the experiment (+26.6%; repeated measures ANOVA, time: F=44.65, p<0.0001), while those clones whose haplotype shifted (i.e. changes at ≥ 2 loci) maintained constant cell densities (-14.5%; repeated measures ANOVA, time: F=4.26, p=0.1311). In response to heat stress, clones whose *Symbiodinium* haplotype changed lost more

than 75% of their *Symbiodinium* (-76.5%, repeated measures ANOVA, time: F=77.35, p=0.0031), and colonies whose haplotype remained the same lost nearly half (repeated measures ANOVA, time: -46.8%, F=231.00, p<0.0001)

The quantum yield of PSII (F_v/F_m) changed by treatment over the course of the experiment (Figure 5.9; repeated measures ANOVA, time: F=90.62, p>0.0001, time*treatment: F=22.90, p<0.0001). Quantum yield remained unchanged in the control group (F=0.82, p=0.3734), but decreased significantly in the groups treated with light or heat stress (light stress: F=30.81, p<0.0001; heat stress: F=58.01, p<0.0001), indicating that the heat and light treatments resulted in significant photoinhibition and/or photodamage. Quantum yield varied significantly among treatments (Tukey's HSD), with heat stress showing a greater decrease in F_v/F_m than light stress (Figure 5.9).

DISCUSSION

The discovery of substantial genetic and ecological diversity among *Symbiodinium* has raised the question whether this diversity might provide corals with additional adaptive flexibility in the face of rapid environmental change. Previous studies of intracolony *Symbiodinium* populations have found that most corals do not change their symbionts over time or in response to environmental stress (Goulet 2006). However, the large majority of these studies were based on coarsely resolved *Symbiodinium* clades, allowing the possibility that symbiont change may commonly occur at finer-scales of diversity. Here we survey the *Symbiodinium* diversity within individual colonies of the Caribbean coral *Gorgonia ventalina*, using hyper-variable microsatellite markers that likely resolve individual clones. We demonstrate that, while most *G. ventalina* colonies maintained a consistent *Symbiodinium* population in the face of light stress, heat stress, and transplant across depth, the symbiont type of

some colonies did change. These results suggest that fine-scale *Symbiodinium* diversity may correspond with functional diversity, and some colonies of *G. ventalina* may be able to "adapt" to environmental change by switching the fine-scale composition of the symbionts they host.

Seasonal Survey

Symbiodinium abundance in marked *G. ventalina* colonies in the field fluctuated seasonally, with cell densities in February as much as 88.5% greater than those in October (Figure 5.3). These results are consistent with studies of a number of other coral species, which show higher *Symbiodinium* densities in months when day length is short and temperatures are low (Stimson 1997, Fagoonee et al. 1999, Fitt et al. 2000, Lasker 2003, Pillay et al. 2005). The fluctuation in cell density is likely driven by two interrelated phenomena. In the summer months, when irradiance is high, the photosynthetic output of *Symbiodinium* is at its maximum, resulting in greater per capita productivity but also increased potential for oxidative damage to the host. Conversely, during the winter months when irradiance is low, productivity is diminished, and greater numbers of *Symbiodinium* may be necessary to meet the host's metabolic demands. There was no significant effect of size class on symbiont abundance, indicating that colonies of varying size all experienced similar seasonal fluctuations.

Despite the dramatic fluctuations in cell density, the large majority of *G. ventalina* colonies maintained a constant symbiont type throughout the year, with the exception of the smallest colonies. *Symbiodinium* haplotype changed in 30% of *G. ventalina* colonies in the smallest size class (≤10cm) over the course of one year, while haplotypes for all colonies larger than 10 cm remained identical throughout the year. As the observed *Symbiodinium* changes were restricted to only the smallest

colonies and did not appear to be seasonally cyclical, these changes do not provide strong evidence for functional symbiont shifts in response to environmental change. Rather, the observed pattern is more consistent with reduced symbiotic specificity of juvenile colonies relative to adults. Coffroth *et al*. (2001) described such a pattern for *Symbiodinium* hosted by two species of Caribbean octocoral. Newly settled polyps of the corals *Plexaura kuna* and *Pseudoplexaura porosa* hosted multiple clades of *Symbiodinium*, yet after just three months of development, these colonies hosted only a single common clade (clade B). Although the smallest *G. ventalina* colonies in this study were all larger and likely older than the colonies surveyed by Coffroth *et al*. (2001), the level of phylogenetic resolution in our data is much finer than the clade level, and it is possible that the expression of specificity at finer scales of *Symbiodinium* diversity extends further into a coral colony's ontogeny. Such results support a general model of the initiation of specificity in cnidarian endosymbioses, whereby the host initially obtains a diverse and dynamic population of symbionts that are reduced throughout development to a stable, low-diversity symbiont population in the adult colony.

Reciprocal Transplant Experiment

Forty-five percent of *G. ventalina* colonies transplanted from shallow to deep and 41.2% of colonies transplanted from deep to shallow changed *Symbiodinium* haplotype at two or more of the eight microsatellite loci surveyed (Figure 5.4). In contrast, no *Symbiodinium* changes were detected among the control colonies. These changes were not significant, although this was almost certainly due to low statistical power resulting from the few control samples. If the criteria for diagnosing intracolony *Symbiodinium* change is relaxed to include colonies with changes at just one locus, then the changes in both transplant groups become significant. On average,

initial symbiont cell densities were significantly lower in deep colonies than shallow colonies, a pattern previously reported for a number of other coral species (Fitt et al. 2000). Shallow control colonies exhibited trends similar to those evident in the seasonal survey, with significantly higher density in months with shorter days and cooler temperatures (Figure 5.3). The deep control group exhibited a similar trend, though much weaker and not significant, suggesting that the seasonal fluctuation in symbiont abundance is attenuated or absent at the lower depth limit of *G. ventalina*. Interestingly, neither transplant group showed signs of seasonal fluctuation, and cell densities in both groups declined over 4 and 12 months. These reductions may be evidence of the chronic decline of intracolony *Symbiodinium* populations poorly adapted to their transplant environment. Cell densities were not significantly different between transplanted colonies that changed *Symbiodinium* halpotype and those that did not, indicating that fine scale symbiont change did not occur rapidly enough or impart functional changes substantial enough to restore typical seasonal dynamics over the time scale examined here.

Heat/Light Stress Experiment

More than 10% of *G. ventalina* colonies exposed to six weeks of heat or light stress changed *Symbiodinium* haplotype (Figure 5.7). These changes differed significantly from clonal control colonies, none of which changed symbiont type. The quantum yield of photosystem II (F_v/F_m) declined significantly in response to both heat and light stress (Figure 5.9), indicating that the *Symbiodinium* in the experimental groups experienced photoinhibition and/or photodamage relative to controls. There were no significant differences between the quantum yield of colonies that had changed *Symbiodium* haplotype and those that had not, indicating that the observed symbiont change had no detectable effect on the photosynthetic response to stress.

However, intracolony *Symbiodinium* change was associated with a reduction in symbiont abundance. In both heat and light stress groups, cell densities were lower in *G. ventalina* colonies that changed *Symbiodinium* haplotype than colonies that did not change (Figure 5.8). This pattern is consistent with aspects of the adaptive bleaching hypothesis, which posits a substantial reduction in symbiont cell density is necessary to disrupt the numerical dominance of the abundant symbiont type(s), allowing either new symbiont types to enter the host or rare types already present at low densities to expand their populations (Buddemeier and Fautin 1993, Ware et al. 1996, Kinzie et al. 2001). It is difficult to conclude that the observed symbiont changes were truly "adaptive" without evidence of functional changes for the holobiont, and at least partial rebounds in cell density would be expected if the new symbiont type were better suited to the new environment. It is possible that the length of this experiment was insufficient to capture such changes, or that functional changes would be evident only after recovery and/or exposure to subsequent stress events. It is noteworthy, however, that mortality was higher among heat stressed *G. ventalina* colonies that did not change *Symbiodinium* haplotype (5/30, 16.7%) than among those colonies that did change symbionts (0/4, Figure 5.7), suggesting that symbiont change may be associated in some way with resilience to environmental stress.

There are several lines of evidence to suggest that the fine-scale intracolony *Symbiodinium* changes observed here may have been due to a redistribution of existing diversity rather than the acquisition of new symbiont types from the environment. In the transplant experiment, although Bayesian clustering analysis identified two distinct *Symbiodinium* populations segregated almost completely by depth, transplanted colonies that changed symbiont type did not acquire symbionts common to their new environment. That is to say, deep corals transplanted to a shallow environment did not obtain symbionts from the "shallow population," and

visa versa. In aquarium experiments, most colonies that changed symbiont type in response to heat or light stress hosted multiple strains of *Symbiodinium* at the start of the experiment. Moreover, it seems unlikely that a large population of *Symbiodinium* would have been available in the artificial seawater environment of the experimental aquaria. While it is probable that some *Symbiodinium* were present due to loss from other colonies, *Symbiodinium* generally exist at much higher density in sediments than the water column (Littman et al. 2008), and there was no sediment available in the aquaria. In addition, the regular water changes in the experimental tanks would also have helped keep water-born *Symbiodinium* densities low. The existence of multiple *Symbiodinium* types at low levels is possible, even within colonies where only a single haplotype is observed. PCR-based surveys can be quite sensitive to template concentrations and tend to preferentially amplify more abundant targets when multiple templates are present. Santos *et al*. (2001) have found that molecular techniques often fail to detect low frequency haplotypes *in hospite*. Indeed, the microsatellite primers used in this study routinely failed to detect low frequency haplotypes in mixed-template reactions at relative frequencies lower than 0.1. This implies that cryptic *Symbiodinium* diversity can exist undetected at substantial frequencies, and may be the source of "new" haplotypes resulting from symbiont switching.

CONCLUSIONS

The results presented here show that fine-scale *Symbiodinium* diversity occurs among and within individual colonies of *G. ventalina*, and that symbiont types can switch in response to environmental change. However, the functional significance of the observed intracolony *Symbiodinium* changes is unknown. Symbiont change did not confer any detectable difference in photosynthetic performance of experimentally stressed corals, and symbiont cell densities among *G. ventalina* colonies that had

changed their symbionts were as low or lower than those colonies whose symbionts did not change. However, the fact that symbiont populations changed at all in response to environmental stress suggests that these changes have some functional significance, and mortality was higher among heat-stressed corals that did not exhibit a change in symbiont type. Longer-term studies of experimentally-stressed corals could resolve whether fine-scale symbiont switching confers upon the coral holobiont functional differences and increased resilience to change.

Although some switching of symbiont types was observed, most *G. ventalina* colonies host only a single detectable clonal type of *Symbiodinium*, and that type did not change over time or in response to dramatic environmental change. While most coral species have been observed to not change their symbionts, the genetic markers used in this study provide much higher resolution than any previously reported longitudinal study of intracolony *Symbiodinium* diversity. These results imply that, even if fine-scale symbiont change does provide some *G. ventalina* colonies with additional adaptive flexibility, such change may be relatively rare and of limited ecological significance.

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